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Editorial Views and News

In this last number of Volume 40 of the *Journal*, it is important to mention that currently there have been developed several significant symposia and workshops concerned with oral area problems. However, unless the contents of their pointed discussions are duly recorded and indexed, the full value of these specific meetings for the benefit of research, teaching, and practice will be very limited.

In 1962, it is planned to publish several supplements to the *Journal of Dental Research* containing the main presentations of such symposia. In other instances, mere reference will be made for the literary record, of worthwhile symposia or workshops where significant topics have been covered. On the remainder of this page, there is published the essence of one such gathering.

WORKSHOP ON ADHESIVE RESTORATIVE DENTAL MATERIALS

A research area indubitably deserving an extremely high priority for further investigation is that concerned with the development of a restorative material or cementing substance which will securely adhere to tooth structure.

To explore current knowledge and to establish recommendations for future fruitful research, an initial workshop was held on September 28 and 29, 1961, at Indianapolis, Indiana. The plans for the workshop originated in the Dental Study Section, Division of Research Grants, National Institutes of Health, and the workshop was sponsored through a grant from the National Institute of Dental Research, U.S.P.H.S., to Indiana University. There were twenty-eight participants, as well as observers from the United States Public Health Service, the United States Armed Forces, and from the A.D.A. Council on Dental Research.

The discussion of *Tooth Structure* was based upon papers by Walter C. Hess, Ph.D., Aron S. Posner, D.Sc., David B. Scott, D.D.S., M.S., Walter E. Brown, Ph.D., and Edward F. Mellon, Ph.D., under the chairmanship of Gerhard M. Brauer, Ph.D.

Principles of Adsorption and Adhesion were analyzed and interpreted by Frederick R. Eirich, Ph.D. (Section Chairman), Ralph A. Beebe, Ph.D., F. Villalonga, Ph.D., Albert C. Zettlemoyer, Ph.D., and W. A. Zisman, Ph.D.

Materials and Test Methods were reported by John A. Cornell, M.S., Michael G. Buonocore, D.M.D., M.S., Ray L. Bowen, D.D.S., Kwan C. Tsou, Ph.D., and others.

The exchange of knowledge and ideas by these authorities in their respective fields pointed up critical areas in which basic knowledge is deficient. These discussions lead to a number of specific recommendations indicative of the direction of future research. These were the four recommendations:

1. There is a need for increased knowledge of the tooth surface, since it is enamel and dentin to which an intimate union must be attained. Additional information regarding apatite chemistry, the mechanism of recrystallization, and formation of secondary dentin would be most helpful.

2. The possibility was suggested that an actual chemical bonding may not be essential and that forces of adsorption might be quite adequate for attaining the intimate relationship desired between the restoration and the tooth. It was the opinion of the experts in the area that moisture in the tooth is the greatest deterrent to securing adhesion. Thus future research must include studies on how to wet a tooth surface and maintain that wetting. In this concept it was also pointed out that true adhesion can best be achieved only on very smooth surfaces which are free of debris, since small bubbles or discrepancies in the film destroy the adhesion.

3. A number of resin systems for bonding with both the organic and inorganic portions of the tooth were discussed. It was the general consensus that greater possibilities lay in bonding to the inorganic structure of the tooth and that future work should probably be beamed in this direction. In these studies it was emphasized that the geometry of the entire system must be taken into consideration. Among other things this would include volume shrinkage occurring during polymerization of resinous materials and the form of the cavity preparation in order that stress at the junction of the tooth and restorative material could be minimized.

4. All present agreed that there is a definite need for the development of better and standardized test procedures for assessing adhesion. The feasibility of developing a standardized surface for testing adhesion was discussed along with the merits of marginal penetration tests and investigations of the strength of surface films.

In general, the conclusions of the workshop indicate that, while there are many obstacles to the development of adhesive restorative material, the problems are definitely not insurmountable.

Proceedings of the workshop were transcribed and will be published. Requests for copies should be directed to the chairman of the workshop, Ralph W. Phillips, Indiana University School of Dentistry, or to the co-chairman, Gunnar Ryge, Marquette University School of Dentistry.

FRANK J. ORLAND
Editor

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

FORTIETH ANNUAL MEETING, MARCH 15, 16, 17, AND 18, 1962
SHERATON JEFFERSON HOTEL, ST. LOUIS, MISSOURI

As in other years, there will be a joint Scientific Session
with the American Association of Dental Schools
on Sunday, March 18

Studies on Dental Plaque. I. The Effect of Different Concentrations of Glucose on the pH of Dental Plaque in Vivo

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Previous investigators[1–8] have found that glucose and other carbohydrates, such as sucrose, are readily fermented with the production of acid by the many organisms present in the plaques adhering to the more secluded surfaces of the teeth. Because foodstuffs vary widely in their (1) carbohydrate content, (2) tendency to be retained on tooth surfaces, (3) tendency to be broken down by salivary enzymes, and (4) in their capacity to stimulate the flow of saliva, their variable effect on salivary sugar levels[9–11] and on plaque pH's[12–15] following their ingestion, would not seem to be too surprising. In spite of these and other numerous variables, the two main factors determining the supply of carbohydrate substrate to the bacteria in a given dental plaque would seem to be (*a*) the concentration of the substrate available and (*b*) the time that any given concentration is present. Since carbohydrate foodstuffs are very likely broken down to the simpler sugars before they are utilized by the oral bacteria and because of its importance in cell metabolism in general, the monosaccharide glucose was the carbohydrate selected in the present investigation to study the effect of these two main factors on plaque pH levels.

EXPERIMENTAL METHODS

Experiments were first carried out in vitro to determine the effect that varying the concentration of glucose in saliva-glucose mixtures might have on the pH produced by the organisms in these mixtures when incubated at 37° C. This was done by setting up in each experiment a series of tubes (in duplicate) each of which contained sufficient glucose in 3 ml. of distilled water that when 2 ml. of freshly collected fasting (i.e., prebreakfast), paraffin-stimulated saliva was added to each, the final glucose concentrations in the mixtures ranged from 0 to 30 per cent. One duplicate of each pair of tubes in the series was then immediately stoppered, leaving only a small air space above the surface of the incubation mixture. Before the corresponding duplicate was also plugged, its pH was measured with a glass electrode and a Cambridge bench-type pH meter. All the mixtures in each of the three experiments as shown in Figure 1 were then incubated at 37° C., two experiments for 24 hours, and the third for 4 hours, after which the pH value of each mixture was determined.

The larger portion of this study was carried out and submitted as part of a thesis for the degree of Doctor of Philosophy in the Department of Physiology, King's College, University of Durham, England, and was then completed at the University of Manitoba with the support of a grant from the Associate Committee on Dental Research, National Research Council of Canada.

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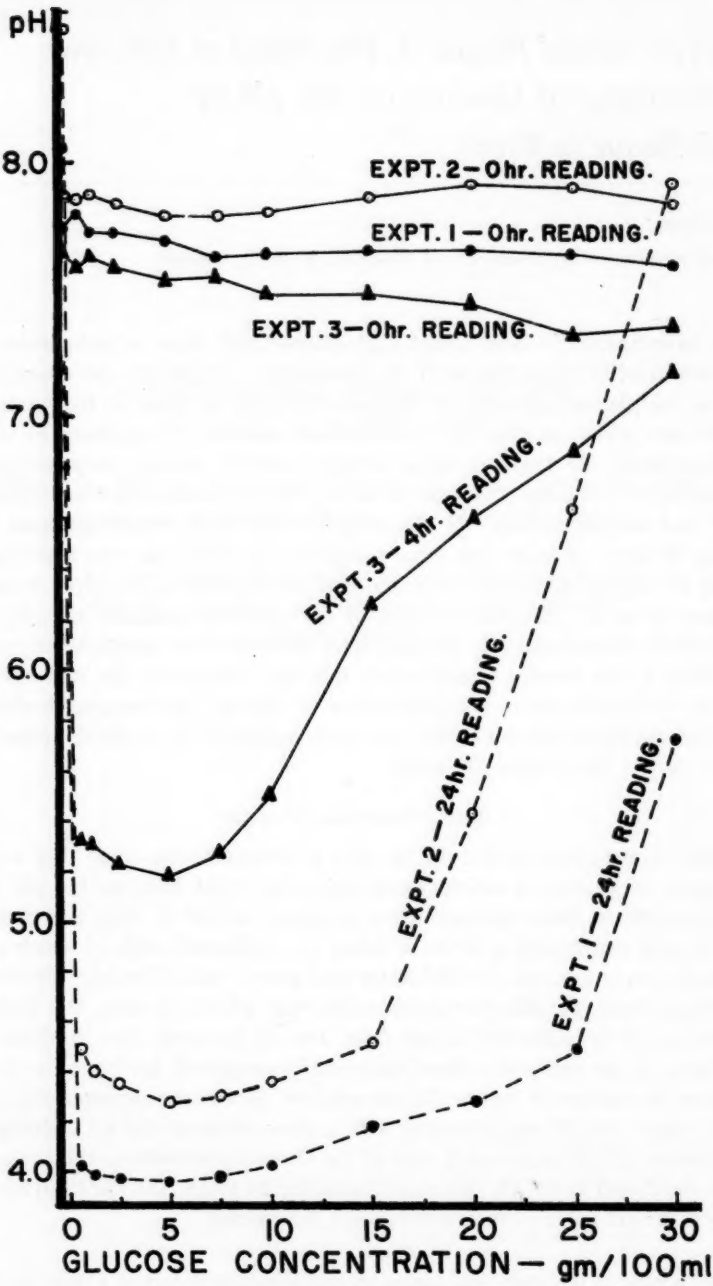

FIG. 1.—The effect of different concentrations of glucose on the pH of incubated saliva-glucose mixtures.

The results from these experiments were then used as a guide in subsequent in vivo experiments in which an attempt was made to determine the effect that adding glucose solutions of different concentrations, for different lengths of time, would have on the pH of selected dental plaques. The time intervals over which these additions were made varied from only a few seconds to as long as 2 hours. By means of a 1-ml. glass syringe to which was fitted a glass nozzle with a very fine tip, small amounts of the glucose were added locally to the plaques that were being studied in each experiment. This was done so that stimulation of saliva flow (which would occur if larger quantities were introduced such as in a rinse or a foodstuff) could, as much as possible, be avoided, especially since it has been found that alterations in saliva flow can alter plaque pH values fairly easily.[12,14,15] Unless otherwise stated, where only a single addition was made the quantity added was allowed to remain on the particular plaque surface for approximately 1 second before the subject's lip was lowered and his mouth closed.

In all cases (except in the studies reported in subsection F), two maxillary anterior interproximal plaque areas were studied, and their pH's were determined with the antimony microelectrodes and procedure previously described.[16] Before all experiments, the subjects were instructed to stop brushing their teeth for 3 days prior to and also on the day of the experiment. The subjects were also instructed not to have any breakfast that day, and the experiments were carried out in each case at approximately the same time each morning.

RESULTS

A. *The effect of different concentrations of glucose on the pH of incubated saliva-glucose mixtures.*—The pH's of the saliva-glucose mixtures before incubation in each of the three experiments shown in Figure 1, varied only slightly, whereas after incubation the variation was considerably greater. Where glucose was not added, the pH of the saliva in each case rose above the initial pH, whereas, in those tubes to which glucose was added, the pH fell. As the concentration of glucose progressively increased in the series, the final pH decreased at first until, at a concentration of 5 per cent, a minimum was reached. With glucose concentrations above 5 per cent, the final pH values increased progressively. This was true for each experiment, although different saliva samples were used. In experiment 3, the pH readings were taken after 4 hours' incubation to rule out the possibility that at the lower concentrations the glucose had been used up. This was easily confirmed by incubating the duplicate tubes with the glucose at the final concentration of 0.6 per cent for a longer period, and after 18 hours the pH's were found to be similar in range to those in the other experiments, viz., 4.52 and 4.56. It might be added that similar observations have been consistently obtained in conjunction with experiments of another study (in preparation for publication).

B. *The effect of the duration of application of a particular concentration of glucose on plaque pH's in vivo.*—The effect of adding a given glucose concentration for short and for longer time intervals is illustrated in the following pair of experiments, in one of which 1 per cent glucose was used, while in the other 0.5 per cent was used.

In the experiment in which one per cent glucose was used, after the initial fasting pH values were determined, a single addition of 0.06 ml. of 1 per cent glucose was

made to each of the two interproximal areas studied. Then, 30 minutes, 60 minutes, and 90 minutes thereafter, a single addition, 5 single additions in sequence at 1-minute intervals, and 10 single additions, also at 1-minute intervals, of 0.06 ml. of the 1 per cent glucose solution were administered respectively to each plaque area (see top of Fig. 2). The pH values were determined at regular intervals throughout, and the results are given in Figure 2. In this figure, the curve obtained with the second single addition of the 1 per cent glucose has been superimposed for comparison (as shown by the dotted lines) on the curve obtained with the first single addition. Furthermore, the curves obtained with both the 5 and the 10 individual additions of the 1 per cent glucose have been superimposed in the same diagram over the curve obtained with the second single addition.

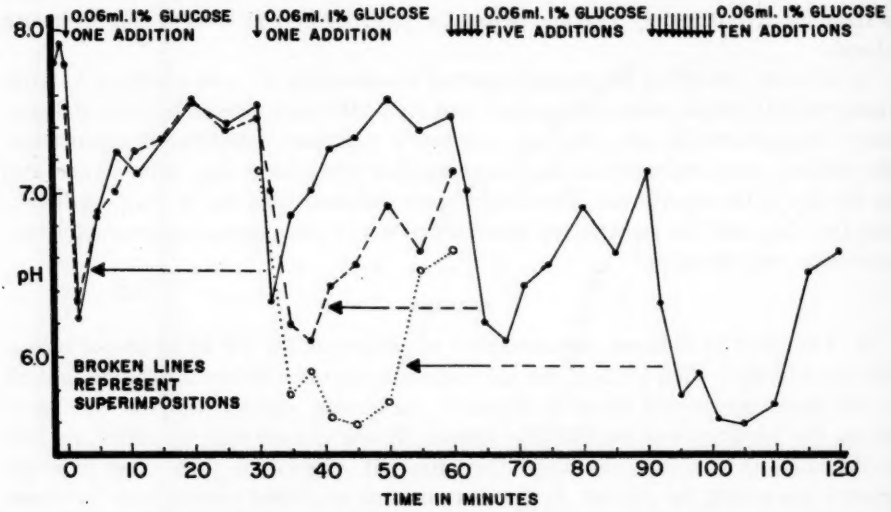
0.06ml. 1% GLUCOSE ONE ADDITION
0.06ml. 1% GLUCOSE ONE ADDITION
0.06ml. 1% GLUCOSE FIVE ADDITIONS
0.06ml. 1% GLUCOSE TEN ADDITIONS
8.0
7.0
6.0
pH
BROKEN LINES REPRESENT SUPERIMPOSITIONS
0 10 20 30 40 50 60 70 80 90 100 110 120
TIME IN MINUTES

FIG. 2.—The effect of adding single and multiple doses of 1 per cent glucose on plaque pH's

Repeating the single glucose application gave a curve approximately similar to that found with the first single addition (Fig. 6), which is in agreement with what had been found previously in numerous preliminary experiments and earlier by both Stephan and Miller[3] and Stralfors[5] in their studies with duplicate glucose rinses. In the 30-minute interval, the pH had not quite returned to the fasting level in either case, and in later experiments this applied even for longer periods. Since the amount of glucose required to cause this pH difference *in this pH range* is comparatively small (see below), so that when added to the second addition it would alter its concentration insignificantly, and since the time required to return to fasting levels can be longer than 3 hours,[15] a return of pH curves to fasting levels was therefore not considered always necessary or practicable for many of the studies carried out in the present investigation. This appeared to be borne out in the studies that follow.

The 5 individual additions gave a larger pH fall, and in the 30-minute interval the pH had not returned to the same level as with the single addition. The 10 additions gave an even greater fall, and the area above the curve was greater than for the 5 ad-

ditions, which, in turn, was greater than for the single additions. These observations seem to indicate that the greater the amount of glucose applied, the greater the pH disturbance and the greater the area above the curve. In the present instance, since the glucose concentration is approximately constant, the area above the curve depends upon the duration of the application of the glucose, as is also clearly shown in the following experiment with 0.5 per cent glucose and in later experiments in which glucose was added continuously over an extended period of time.

In the experiment in which 0.5 per cent glucose was used, a single addition of this concentration was first made to the interproximal areas studied, and the pH values, before and after this and subsequent additions, were determined at regular intervals. Following the pH curve that resulted, additions of the same quantity of glucose (i.e., 0.06 ml. of 0.5 per cent) were then made at each 2-minute interval for a period of approximately 30 minutes, after which no further additions were made for another

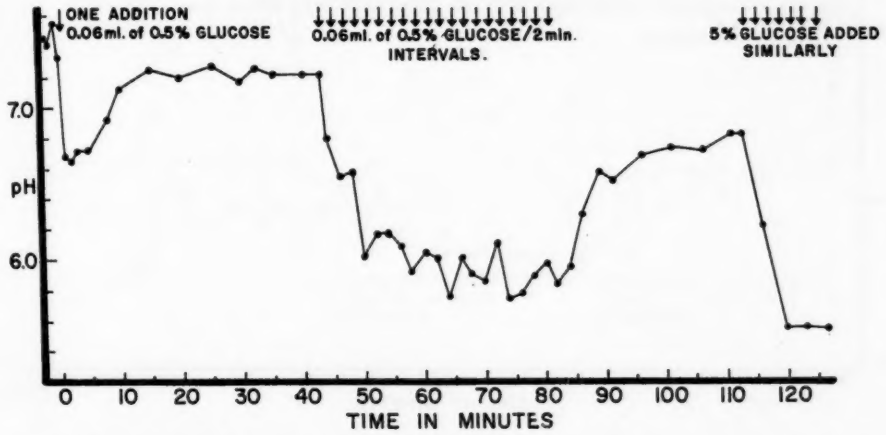

FIG. 3.—The effect of adding single and multiple doses of 0.5 per cent glucose on plaque pH values

30 minutes. To determine whether the pH minimum had been reached during this procedure, 5 per cent glucose, which in the in vitro experiments showed maximum acid production, was then added continuously. The results are given in Figure 3.

With the single glucose addition first made, it was found that the pH fell rapidly from an average initial value of 7.44 to a minimum value of 6.63 and then rose, at first rapidly and later more slowly toward the fasting level. With similar quantities of glucose added continuously, the pH dropped farther than with the single addition and maintained an approximately constant level. When the additions of glucose were no longer made, however, that is, when the glucose "stimulus" was removed, the pH rose as before, at first rapidly and then more slowly, toward the original level. The lowest pH found with the 5 per cent glucose was 5.54, while that with 0.5 per cent was 5.74.

C. *The effect of different concentrations of glucose on plaque pH values with the time of application held constant but of relatively long duration.* 1. *A comparison of 0.1 per cent, 5 per cent, and 50 per cent glucose.*—Lanke[11] studied the effect of time

on the concentration of sugar in the salivas of different individuals after eating various carbohydrate foods. Because she used a concentration of 1 mg/ml (i.e., 0.1 per cent) as a reference level in these important studies, this concentration was selected in the present and later experiments as an example of a relatively low glucose concentration. In a manner similar to that used in previous experiments, the concentration of glucose was maintained approximately constant for 30 minutes to two upper anterior interproximal areas by the addition of 0.06-ml. quantities of 0.1 per cent glucose at regular 1-minute intervals. This 0.1 per cent glucose "stimulus" was then immediately followed by two further 30-minute periods of repeated 1-minute "stimuli," the first with a glucose concentration of 5 per cent and the second with a concentration of 50 per cent. The pH values for the two plaque areas studied were again determined before, during, and at intervals after these stimuli. The results are given in Figure 4.

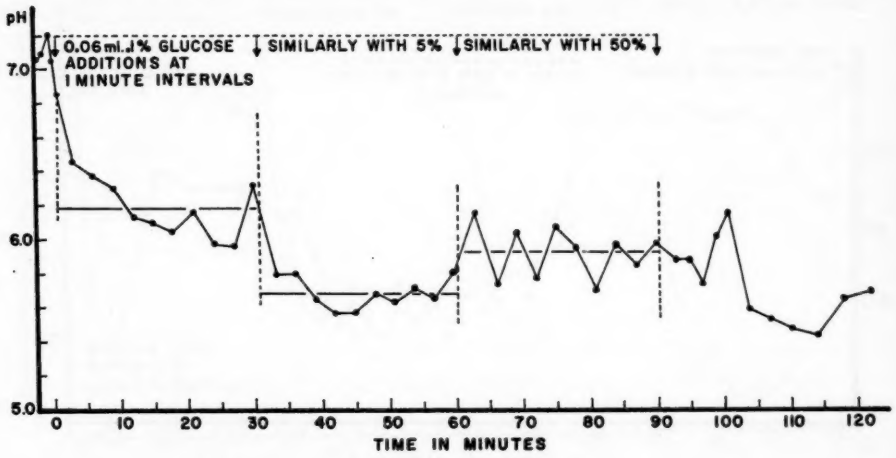

FIG. 4.—Comparison of the effects on plaque pH levels of the application of 0.1, 5, and 50 per cent glucose, when each is applied for a 30-minute period.

It was found that with the 0.1 per cent glucose there was an appreciable drop in pH of approximately 1 pH unit, with the pH dropping approximately 0.5 pH units lower still when the 5 per cent glucose was introduced. With the 50 per cent glucose, the pH level rose, suggesting that some inhibition of acid production had occurred, as in the in vitro experiments above with the higher glucose concentration, but the effect in vivo was of less magnitude. It is rather interesting that this inhibition continued for a short period after the 50 per cent glucose additions were stopped and that the pH dropped before it subsequently rose. Since the diffusion of glucose into plaque material, as found by Muntz and Miller,[17] is a relatively slow process, when the 50 per cent glucose solution was discontinued, it is very likely that the diffusion of the glucose out of the plaque was also relatively slow and that high, but slightly inhibitory, concentrations of glucose were still present to continue the inhibition. This explanation is supported by the observation that a fall in pH occurred before the usual rise. That is, as the glucose continued to diffuse, the concentration in the plaque fell to more suitable levels for acid production, and the pH therefore fell. Then, as the

glucose concentration in the plaque fell still further, less and less substrate became available for the plaque bacteria, and, with the decreasing acid production as a consequence, the pH rose in the usual way. This observation is also shown in a similar type of experiment in subsection E, below.

2. *The effect on plaque pH values of concentrations of glucose less than 0.1 per cent (i.e., 0.01 and 0.005 per cent).*—In the present experiments, a glucose concentration of 0.01 per cent was used, in the same manner as above, for the purpose of determining whether this concentration would have any appreciable effect on the fasting pH levels of the bacterial plaques of the two subjects studied. In subject A, fasting pH

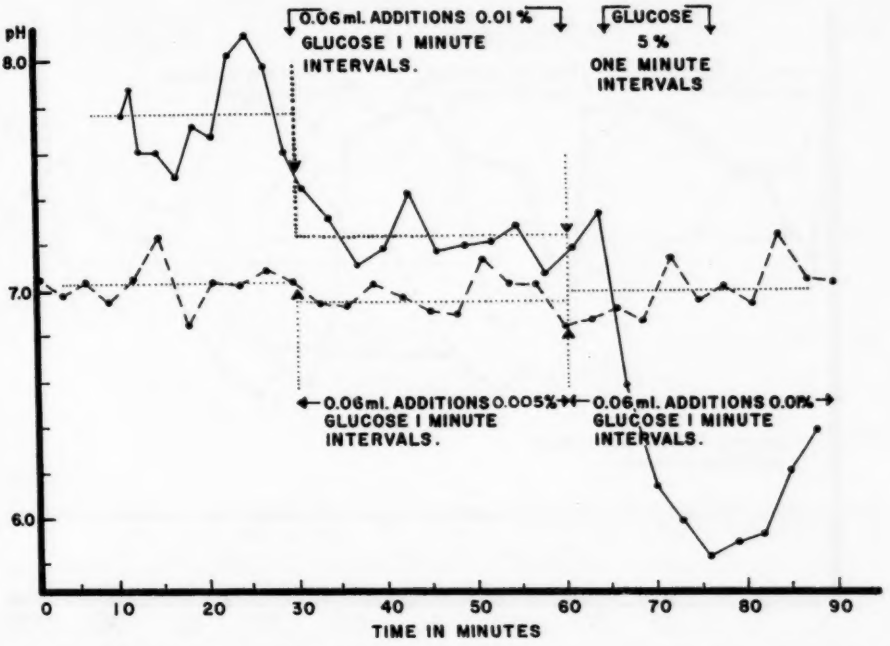

FIG. 5.—The effect on plaque pH levels of comparatively low concentrations of glucose—0.005 and 0.01 per cent.

values were first determined for a 20-minute period, after which 0.06-ml. additions of 0.01 per cent glucose were made, as before, at 1-minute intervals for a period of 30 minutes. After a short delay of 5 minutes, the 0.01 per cent glucose additions were replaced by similar additions of 5 per cent glucose for a period of 12 minutes to determine the pH minimum. The pH's were determined throughout, and the results are given in Figure 5. In subject B, the fasting pH values were first determined for a period of 30 minutes. Following this, 0.06-ml. additions of 0.005 and 0.01 per cent glucose were made at 1-minute intervals for 30 minutes for each of these concentrations, and the pH values were determined at intervals throughout. The results are given in Figure 5, together with the results for subject A.

In subject A, the mean fasting pH value was 7.77, and this dropped to a mean pH value of 7.23 when the 0.01 per cent glucose was added. The 5 per cent glucose caused

a further drop in pH to a minimum of 5.81, and the pH then rose after these additions were stopped. With subject B, however, the plaque pH values remained practically unaltered when either the 0.005 per cent or the 0.01 per cent glucose was added, which might suggest that the substrate for the acid-producing bacteria was present in the saliva at a concentration approximately equivalent to a glucose concentration of 0.01 per cent.

The reasons for these differences in subjects have been considered elsewhere;[15] the important point to consider here is that low glucose concentrations gave a small plaque pH fall.

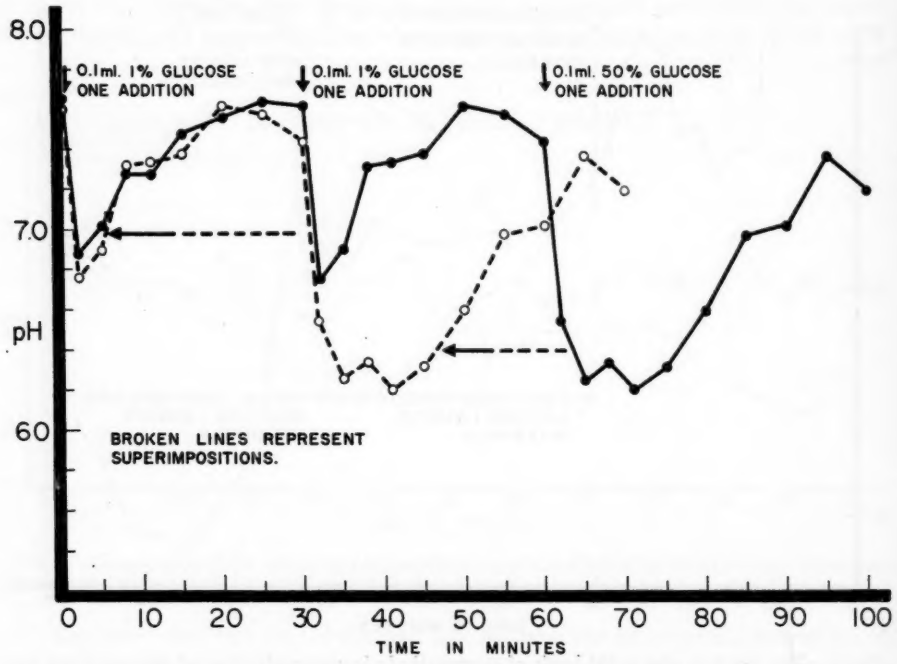

FIG. 6.—Comparison of the effects on plaque pH levels of single additions of 1 and 50 per cent glucose.

D. *The effect of different concentrations of glucose on plaque pH values with the length of time of application constant and of short duration.* 1. *A comparison between the effects of 1 and 50 per cent glucose.*—After the initial fasting pH values were determined, a single addition of 0.1 ml. of 1 per cent glucose was made to each of the two interproximal areas studied. Then, at 30 minutes thereafter, another single addition of 0.1 ml. of 1 per cent glucose was made, while at 60 minutes thereafter a similar addition of 50 per cent glucose was made. The pH readings were taken at regular intervals throughout, and the results are given in Figure 6. It can be seen that the duplicate additions of 1 per cent glucose gave similar curves, whereas the 50 per cent glucose addition gave a greater pH fall and a greater area above the curve.

2. *A comparison between the effects of 5 and 50 per cent glucose.*—In this experi-

ment, after the initial fasting pH values were taken, a single addition of 0.1 ml. of 5 per cent glucose was made to the two areas studied, and the pH changes were followed for a period of 60 minutes. A similar single addition of 50 per cent glucose was then made, and the pH changes were again followed for a period of 60 minutes. In each case, the 0.1-ml. additions were allowed to remain on the plaque surfaces for 10 seconds before the subject's lip was lowered, and he was then instructed to close his mouth. From the results in Figure 7 it can be seen that the 50 per cent glucose gave a greater area above the curve than did the 5 per cent glucose. It should be pointed out that in this type of experiment the 50 per cent glucose actually causes the

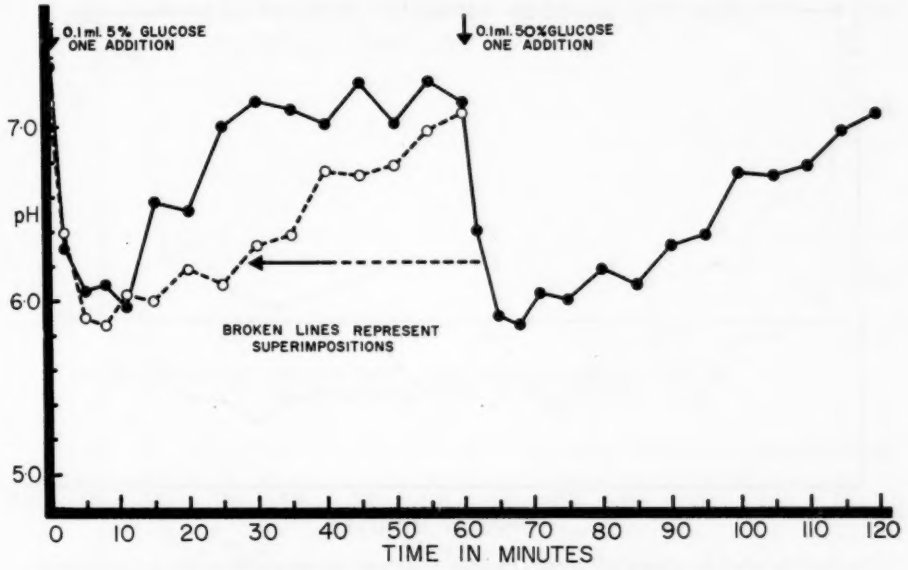

FIG. 7.—Comparison of the effects on plaque pH levels of single additions of 5 and 50 per cent glucose.

pH to fall to as low or a lower pH value than the 5 per cent glucose—an observation which is in contrast to the experiments in subsection C above, in which these same concentrations applied *for longer periods* were compared and gave the reverse results.

It appears from the observations in this subsection that, with glucose applications of short duration, higher glucose concentrations give a greater plaque pH fall than did lower concentrations and that a longer time interval is required for the plaque pH values to return to their original values with the former. An explanation for these results is provided in the discussion below.

E. *The effect of glucose added continuously over an extended period of time, on plaque pH levels in vivo.* 1. *5 per cent glucose.*—To study the effect of maintaining a given glucose concentration to the plaque bacteria at a constant level over an extended period of time, 0.06-ml. additions of 5 per cent glucose were made at 1-minute intervals for 110 minutes. As in previous experiments, the pH values were determined throughout the experiment. The results are given in Figure 8. From these results,

which are confirmed in a similar experiment with 1 per cent glucose which follows below, it would appear that, once a minimum pH is reached, this pH is maintained as long as the glucose is added.

2. *1 per cent glucose.*—This experiment was carried out in the same way as was the previous one with the 5 per cent glucose, except that the additions were carried out for 130 minutes and the pH was followed for 25 minutes after the additions of the 1 per cent glucose were stopped (Fig. 9, *a*). In this experiment, no slight decreasing trend of the pH with time was observed, as seemed to be the case with the 5 per cent additions.

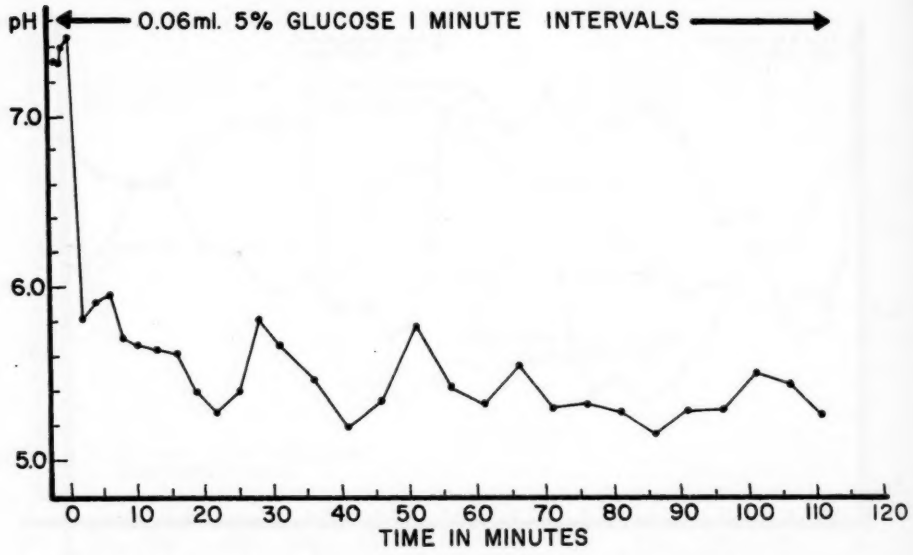

FIG. 8.—The effect on plaque pH levels of adding 5 per cent glucose continuously for 110 minutes

Also included in this subsection are the results of an experiment in which 0.06-ml. additions of 50 per cent glucose at 40-second intervals for 20 minutes were made to plaque areas in the usual way. These results are given in Figure 9, *b*, for comparison with the previous experiment with 1 per cent glucose.

During the 50 per cent "stimulation" period, the pH level reached was approximately 6.20. Then, with the cessation of this "stimulus," instead of the pH rising as with the 1 per cent glucose, it fell to a pH of 5.40 and rose thereafter in the usual way (compare the shape of this curve with that in Fig. 4, and see Fig. 10).

These observations again seem to show that with very high glucose concentrations it is possible to inhibit the pH fall in vivo, but when the glucose concentration is allowed to fall, more suitable concentrations for bacterial acid production are reached, with a resulting drop in pH to a minimum. It is also interesting to note that, with the shorter time of addition of the 50 per cent glucose than that in subsection C above, the pH fell almost immediately on cessation of the additions. This might possibly be because the plaque and the area about the plaque had not had the time to become fully saturated with the 50 per cent glucose, as was the case in the experiment above

with this glucose concentration. However, what the above experiments do illustrate (and the reason that this experiment with 50 per cent glucose is included here) is the tendency for the pH curve to rise after the 1 per cent glucose additions are stopped, whereas after 50 per cent glucose (if it has been added for at least the periods given above), it does not tend to rise immediately and, if anything, tends to fall slightly before subsequently rising (cf. Figs. 4, 6, and 7 above).

F. *A comparison of the maximum plaque pH falls obtained with different glucose concentrations.*—From the results in subsection C, it seems clear that if very low concentrations of glucose are added to plaque, only a small decrease in the pH value

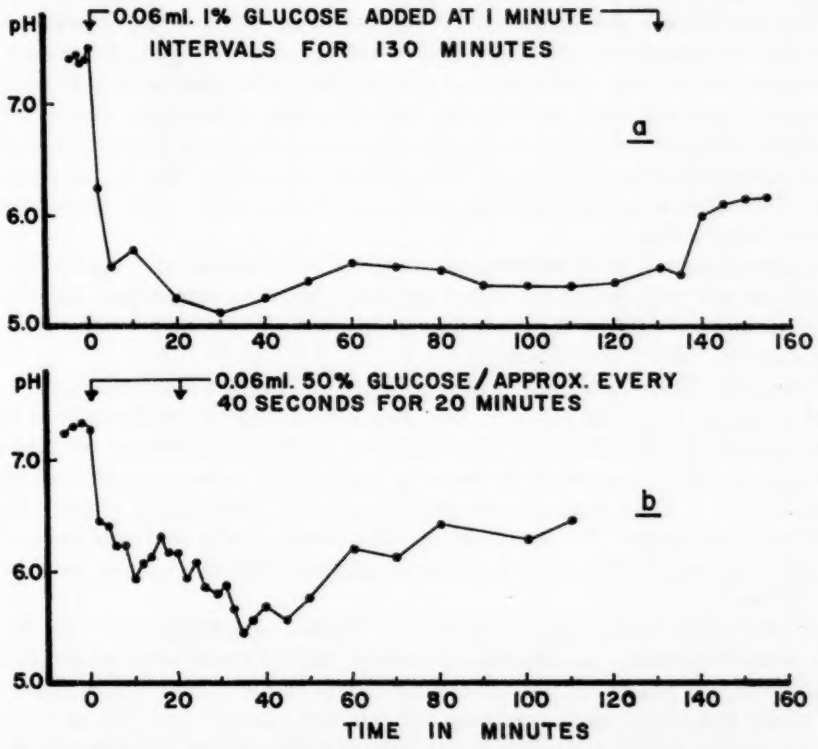

FIG. 9.—*a:* The effect on plaque pH levels of adding 1 per cent glucose continuously for 130 minutes. *b:* The effect on plaque pH levels of the continuous addition of 50 per cent glucose for 20 minutes.

results; with higher concentrations, a larger pH fall was observed, being slightly less with very high concentrations. To check this observation, the effects of different glucose concentrations on selected dental plaques in each of three subjects were investigated and compared in the following way. Several experiments were carried out on each subject, and in each experiment the effects with one glucose concentration were compared with those for 5 per cent glucose. By taking the pH values observed for 5 per cent glucose as giving a maximum or nearly maximum pH fall (and considering this to be a 100 per cent pH drop) and then by expressing the pH drop obtained with the different glucose concentrations tested as a percentage of that obtained with the

5 per cent glucose, it was possible to make comparisons not only between a given glucose concentration and 5 per cent glucose in the same experiment but also between the effects of a number of different glucose concentrations determined on a given individual in different experiments. That is, the difference between the fasting pH value and the pH after 5 per cent glucose would act as a "yardstick" in each experiment.

Two maxillary anterior interproximal and two maxillary labial gingival plaque areas were selected in each of the subjects, and in each case the same selected areas were studied with each glucose concentration tested. After the fasting pH values were determined (5 readings per area), the glucose concentration to be compared with the 5 per cent glucose was added as 0.06-ml. quantities at 30-second intervals for 30 minutes. Following this, the pH values of the plaques were again determined, and, as before, five readings were taken per area. Because of the time required to take these readings, especially since the electrode and salt-bridge were always washed between readings with distilled water and wiped with clean filter paper, the glucose additions were continued during the time that the readings were made. That is, the plaque pH was maintained at a constant level, each level corresponding to the glucose concentration being added.

After an interval of 15 minutes during which no additions were made, this same procedure was then carried out with 5 per cent glucose for comparison, the pH readings being determined in the same manner as above. The results for the 3 subjects are given in Table 1 and Figure 10.

One might digress here for a moment to point out that if a 5 per cent glucose solution is used to bring the pH of a given plaque to or near its minimum level by the above procedure, the effect of a given substance on the metabolism of the acidogenic bacteria in this plaque could be tested by including a given concentration of this substance in a 5 per cent glucose solution and then determining whether the pH minimum was altered. To determine the effectiveness of the substance being tested at pH levels higher than the pH minimum, glucose solutions of lower concentration would have to be used.

In the present study, from the findings in Table 1 and Figure 10, it can be seen (as in the experiments in subsection A, above) that (1) with very low glucose concentrations, the plaque pH fall was directly related to the glucose concentration; (2) at higher concentrations, a maximum pH fall was reached; and (3) at very high concentrations, the pH fall was indirectly related to the glucose concentration. It is of interest that at the higher glucose concentrations the inhibition of the pH fall in vivo was appreciably less than in vitro (cf. Figs. 1 and 10).

It might be pointed out here that the results for the interproximal areas seem to show a higher fasting pH value, a lower pH minimum (i.e., after 5 per cent glucose), and therefore a greater pH decrease than the labial plaques; observations similar to those made in an extensive study on the pH levels occurring in plaques in the different areas of the mouth are reported elsewhere.[15]

It is also of interest that the addition of distilled water (0 per cent glucose) resulted in a pH fall similar to that for 0.01 and 0.05 per cent glucose. From observations reported elsewhere[15] and from unpublished data, this pH fall with the distilled water seems to be related to the relative amounts of nitrogenous and carbohydrate substrates

TABLE 1

Comparison of Maximum Plaque pH Falls Obtained with Different Glucose Concentrations

Subject	Glucose Conc. (Gm. Per Cent)	Interproximal Plaques			Labial Plaques			Interproximal+Labial		
		Mean pH	pH Fall (−Δ pH)	Per Cent pH Fall	Mean pH	pH Fall (−Δ pH)	Per Cent pH Fall	Mean pH	pH Fall (−Δ pH)	Per Cent pH Fall
VS	0	7.82	0	0	7.32	0	0	7.58	0	0
	0*	7.10	0.72	32.(7)	6.92	0.40	20.(3)	7.01	0.57	26.(5)
	5	5.62	2.20	100	5.35	1.97	100	5.49	2.09	100
	0	7.42	0	0	7.15	0	0	7.29	0	0
	0*	6.94	0.48	25.(4)	6.82	0.33	20.(0)	6.88	0.41	22.(7)
	5	5.53	1.89	100	5.50	1.65	100	5.52	1.77	100
	0	7.48	0	0	7.28	0	0	7.39	0	0
	0.01	7.05	0.43	20.(6)	6.50	0.78	42.(4)	6.77	0.62	31.(5)
	5	5.39	2.09	100	5.44	1.84	100	5.42	1.97	100
	0	7.28	0	0	6.75	0	0	7.00	0	0
	0.05	6.78	0.50	25.(3)	6.30	0.45	37.(8)	6.54	0.46	26.(6)
	5	5.30	1.98	100	5.56	1.19	100	5.43	1.57	100
	0	7.16	0	0	6.78	0	0	6.97	0	0
	0.1	6.32	0.84	46.(4)	6.25	0.53	44.(2)	6.29	0.68	45.(3)
	5	5.35	1.81	100	5.58	1.20	100	5.47	1.50	100
	0	7.45	0	0	7.35	0	0	7.40	0	0
	0.5	5.53	1.92	84.(6)	6.10	1.25	93.(3)	5.81	1.59	89.(0)
	5	5.18	2.27	100	6.01	1.34	100	5.60	1.80	100
	0	7.87	0	0	7.66	0	0	7.77	0	0
	10	5.80	2.07	90.(4)	6.13	1.53	85.(5)	5.97	1.80	88.(0)
	5	5.58	2.29	100	5.87	1.79	100	5.73	2.04	100
	0	7.21	0	0	7.19	0	0	7.20	0	0
	30	5.60	1.61	108.(8)	5.92	1.27	87.(0)	5.76	1.44	97.(9)
	5	5.73	1.48	100	5.73	1.46	100	5.73	1.47	100
	0	7.54	0	0	7.36	0	0	7.45	0	0
	50	6.04	1.50	75.(0)	5.93	1.43	76.(1)	5.99	1.46	75.(6)
	5	5.54	2.00	100	5.48	1.88	100	5.51	1.94	100
EM	0	7.40	0	0	7.34	0	0	7.37	0	0
	0.05	7.16	0.24	17.(0)	7.26	0.08	8.(5)	7.21	0.16	12.(6)
	5	5.95	1.45	100	6.40	0.94	100	6.18	1.19	100
	0	7.73	0	0	7.60	0	0	7.67	0	0
	0.5	6.20	1.53	83.(6)	6.31	1.29	112.(2)	6.26	1.41	97.(9)
	5	5.90	1.83	100	6.45	1.15	100	6.18	1.49	100
	0	7.66	0	0	7.42	0	0	7.54	0	0
	50	5.94	1.72	87.(8)	6.07	1.35	86.(0)	6.01	1.53	86.(9)
	5	5.70	1.96	100	5.85	1.57	100	5.78	1.76	100
GL	0	8.00	0	0	7.86	0	0	7.93	0	0
	0.1	7.86	0.14	9.(2)	7.40	0.46	33.(3)	7.63	0.30	21.(3)
	5	6.48	1.52	100	6.48	1.38	100	6.48	1.45	100
	0	8.00	0	0	7.88	0	0	7.94	0	0
	0.5	6.74	1.26	50.(0)	6.76	1.04	60.(8)	6.75	1.15	55.(4)
	5	5.48	2.52	100	6.07	1.71	100	5.78	2.12	100
	0	7.92	0	0	7.62	0	0	7.75	0	0
	10	5.91	2.01	109.(2)	6.25	1.37	116.(1)	6.08	1.67	112.(7)
	5	6.08	1.84	100	6.44	1.18	100	6.26	1.49	100
	0	7.88	0	0	7.69	0	0	7.80	0	0
	30	5.88	2.00	103.(6)	5.73	1.96	107.(7)	5.81	1.99	105.(7)
	5	5.95	1.93	100	5.87	1.82	100	5.92	1.88	100
	0	7.95	0	0	7.72	0	0	7.84	0	0
	50	6.32	1.63	84.(0)	6.43	1.29	81.(5)	6.38	1.46	82.(8)
	5	6.01	1.94	100	6.15	1.57	100	6.08	1.76	100

* Distilled water.

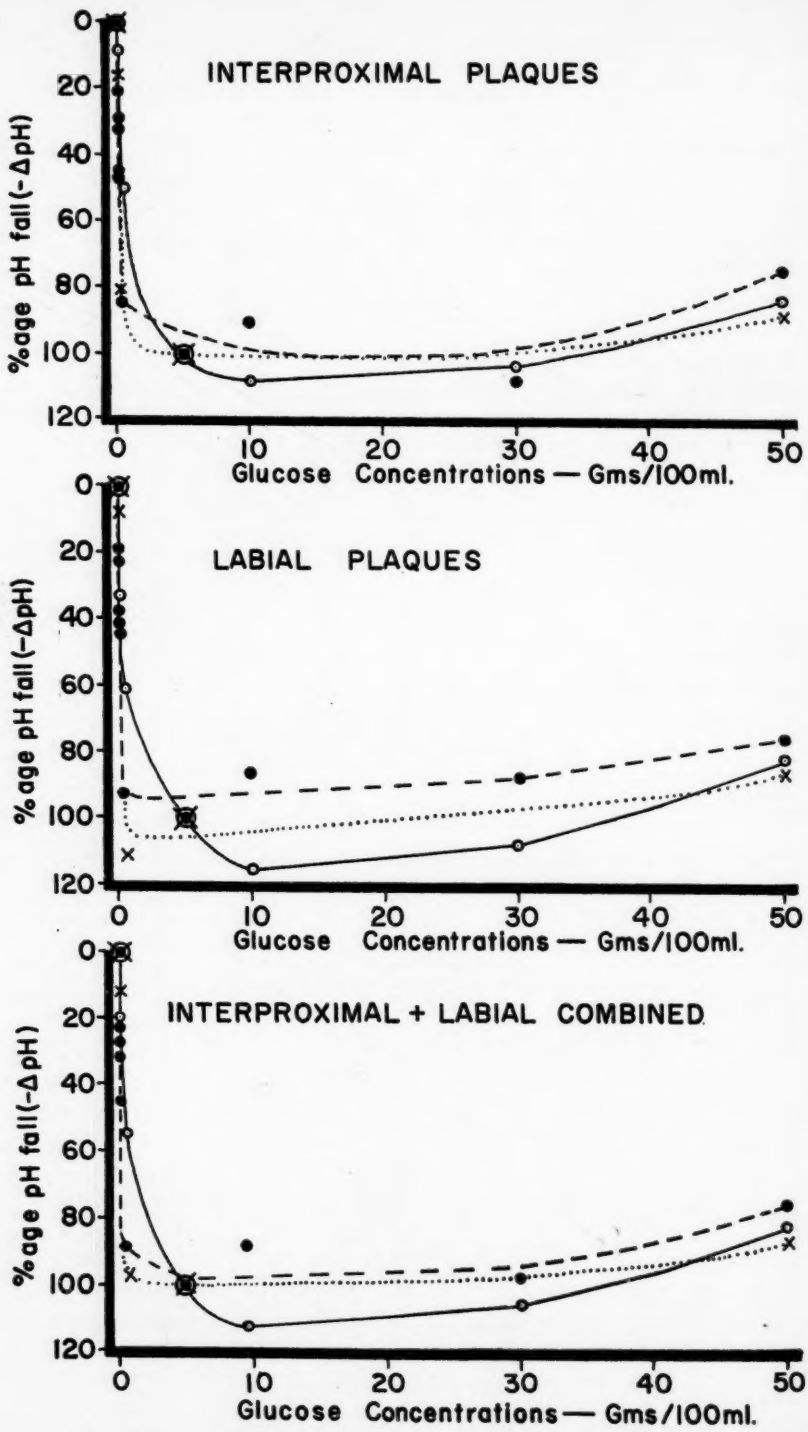

FIG. 10.—The relationship between the glucose concentration and the percentage decrease in interproximal and labial plaque pH levels in each of three subjects, (V.S. ———•———, E.M. .....×...., and G.L. ——— ⊙ ———).

available to the plaque bacteria from the saliva. That is, the water will reduce the concentration of both the nitrogenous and the carbohydrate substrates, and if the former concentration is greater than the latter—say in the fasting state—its concentration will be reduced to a greater extent, thus reducing the alkali-producing tendency of the plaque relative to its tendency to produce acid (see the discussion below).

It should also be pointed out here that if the per cent pH fall is calculated from the pH with 0 per cent glucose (i.e., distilled water) rather than using the fasting pH as the "base line," the results would be essentially similar. Because the fasting pH level is more convenient to determine in each experiment in this subsection and seems to be of some importance in other experiments, it has been chosen here as the base line, although it has been kept in mind that, depending upon what is being studied, one base-line level may be more correct than the other.

DISCUSSION

Before proceeding to discuss the observations made in the experiments in this investigation, it is important to consider initially some of the general properties of the in vitro and in vivo systems studied here, so that a better understanding of their behavior might possibly be obtained. It should first be pointed out that these two systems differ in two main respects: (1) The bacteria in the saliva-glucose mixtures are not highly concentrated in a localized area as are the bacteria in dental plaque but, instead, are widely dispersed throughout a relatively large saliva medium which permits considerable "room" for each bacterium to grow, and (2) the in vitro system is closed and therefore starts with definite quantities of nutrients which tend to decrease to varying degrees with time, while, on the other hand, the in vivo system is open, and therefore the supply of nutrients is continuous and can be either at constant or at variable levels. One feature that is common, of course, in these two systems is that they contain a variety of different types of micro-organisms.

Although the number and types of organisms that are present in the oral flora are extremely large, these organisms will, on the basis of the substrate or substrates that they are able to utilize, be divided into what will be referred to here as the "proteolytic" and "glycolytic" types, the former utilizing nitrogenous materials to give alkaline end-products, the latter utilizing carbohydrate to give end-products that are acidic. It should be pointed out, however, that if a micro-organism can act on both these types of substrates, for the purpose of this "classification" this organism will fall into both groups.

The pH of the saliva-glucose mixtures and the dental plaques studied in the present investigation can be considered to be affected by several factors, although one must, of course, keep in mind that these factors can be expected to be acting simultaneously and that their magnitudes will determine the pH value of the glucose mixture or plaque at any given time. The pH of either system will tend to be lowered by the formation of acids such as lactic from carbohydrate substrates, while the formation of alkaline end-products from nitrogenous substrates and/or the removal of the acids that have accumulated during glycolysis by further metabolism will tend to raise the pH of the particular system in question.[18] In the mouth, because of the presence of saliva continuously flowing by the dental plaque, a strong tendency will exist for the end-products formed during both glycolysis and proteolysis to be rapidly removed by

diffusion. As a result, this property of saliva (as well as its buffering properties) will continuously tend to bring the pH of the plaque closer to its own value; that is, if the plaque pH is more alkaline than that of saliva, the saliva will tend to lower the plaque pH, whereas if the plaque pH were more acid than that of the saliva, its pH would tend to be raised. It should also be pointed out that, since the optimum pH for bacterial metabolism is approximately 0.5 pH units on either side of neutrality, as the pH continues to rise above this range in the absence of glucose or continues to fall below this range in its presence, the pH becomes less favorable for the metabolism of the particular group of bacteria for which these conditions are favorable.[19]

Of these factors, the supply of nutrients to the proteolytic bacteria can be assumed to be approximately constant (the effect of its variation has been considered elsewhere),[15] and, since the studies in vivo were carried out under conditions of minimal salivary stimulation, the effect of the saliva can also be considered to be approximately constant.[12, 14, 15] It must also be realized that in the saliva-glucose mixtures one is starting in each case with a system that may not be initially at equilibrium with respect to pH, and if glucose is not added, the pH can rise on incubation until an approximate "alkaline" steady state is reached. Some of this pH rise is, of course, because of CO_2 loss, but if precautions are taken to prevent its escape, the observations are similar, indicating the tendency for the proteolytic bacteria to convert salivary nitrogenous material to alkaline end-products.

On the other hand, in the in vivo studies, since all the subjects were fasting at the beginning of each experiment, plaque pH values were almost invariably on the alkaline side of neutrality, and the system was already at an approximate equilibrium state. In both the in vivo and the in vitro systems, the tendency is for both systems to be or to become alkaline and to remain at an approximate alkaline equilibrium state unless glucose is added. If, however, glucose is added, the equilibrium state is then disturbed toward a state of greater acidity, and the magnitude and duration of this disturbance are, as seen in the above experiments, dependent on the amount and duration of the glucose added.

Stralfors[5] has shown, from his experimental data and by calculation, that the slow fall in pH in saliva-glucose mixtures, when compared with the very rapid pH change in dental plaque, can be accounted for by the fact that the initial concentration of acidogenic bacteria is lower in the former. In a growing culture medium containing glucose, he found that, as the bacteria in the medium increased in number over the period of incubation, the *total* amount of acid produced also increased but that, as the pH fell as a result of the accumulation of this acid, the actual *rate of acid production* per unit cell fell. The rate of acid production, he found, decreased exponentially with the pH, being approximately 10 times more rapid in the pH range 6.5–7.0 than in the pH range 4.5–5.0. This variation in the rate of acid production with the pH varied, however, with the different bacteria studied. The lactobacilli, for example, had a lesser rate than the streptococci in the higher pH ranges but a greater rate at lower ranges. He also noted that those bacteria that had a shorter generation time also gave a more rapid rate of acid production for a given number of bacterial cells, and, in addition, even for the same bacterial strain the generation time was inversely related to the rate of acid production. He therefore suggested that the explanation for these observations might be that the multiplication process required energy, which, he pointed out, could be

obtained from the splitting of sugar to lactic acid and that, with a high rate of sugar decomposition, more energy would be available to permit a more rapid cell multiplication. To put this in another way, those bacterial cells that could utilize the glucose most readily grew most rapidly, indicating that increased metabolism is associated with increased growth, as is the case when comparing, for example, the young animal with the older one.[20]

Let us now consider these observations and those in the present investigation in terms of the following relationships:

$$\text{Glucose} + \underset{\text{(in the cells)}}{\text{multienzyme units}} \overset{1}{\underset{2}{\rightleftarrows}} \text{acid} \overset{3}{\rightarrow} \text{processes that remove the acid} . \qquad (1)$$

A multienzyme unit is defined here as a given amount of multienzyme system in a bacterial cell and is comparable to enzyme concentration but is defined to include a given amount of the different enzymes in the whole sequence of enzymes that take part in the conversion of a given amount of substrate (in this case, glucose) to product (in this case, acid).

Step 3 above, that is, the processes that remove the acids, will include (1) the further metabolism of the acids to less acidic end products, (2) neutralization of these acids by the alkaline end-products of proteolysis, (3) neutralization by the salivary buffers, and (4) the diffusion of the acids away from the *cell surfaces*. For the reasons given earlier in this discussion, of these, (2) and (3) can be ignored in the studies in this investigation, while (1) and (4) of step 3 and process 2 will be dependent on process 1, and, in turn, these processes can affect the rate of process 1, as will be discussed below. The concentration of acid that is present and consequently the pH of the medium will tend to be the net result of the above processes.

In the saliva-glucose mixtures, the introduction of glucose will result initially in an increase in the number of acidogenic bacteria, and therefore the concentration of enzyme units will rise, so that, even though there is some decrease in the glucose concentration in each mixture during the incubation period, there is a net increase in the rate of process 1. Since process 1 is more rapid at first than processes 2 and 3, acid accumulates, and the pH falls. Because these mixtures are essentially closed systems and the source of oxygen (which would tend to speed up the further metabolism of the acids) is limited (as is also the case in vivo[5]), the rate of process 3 is *comparatively* slow, while any appreciable amounts of glucose are still present.[18] Therefore, as the time of incubation continues, the progressive increase in the concentration of the acids in the mixtures can be expected, because of the slowness of process 3 in this case, to result in a correspondingly progressive increase in the rate of process 2. The acids would also compete with the glucose for the enzyme units, and the rate of process 1 would therefore correspondingly decrease, the net result being a progressive slowing of the actual rate at which the acid is accumulating. Since the bacteria require the energy from process 1 for their survival and growth, as the rate of this process decreases, the net increase in bacterial growth and therefore the rate of change of multienzyme units would also decrease, further contributing to slowing the rate of process 1.

The above appears to explain in the general terms of equation (1) the more important observations made by Stralfors[5] and Stephan and Hemmens.[18] Since the only variable in the different glucose mixtures studied in the present investigation was the glu-

cose concentration, the same principles can be expected to apply. The changes observed can be expected to reflect the net effect of the glucose concentration on the rates of processes 1, 2, and 3.

In vivo, however, process 3, because of the continuous flow of saliva, will tend to remove any acid produced by the plaque bacteria fairly readily. Because acid cannot accumulate as easily as in vitro, however, process 2 becomes comparatively small and is the reverse of the situation found in vitro. In addition, in vivo, the enzyme units are present initially at a high concentration, since there is a high concentration of cells, and will not be expected to alter appreciably in these experiments (this will be commented on below). Further, since the glucose can be removed by the saliva, its availability to the bacteria will be more variable than in vitro. Since, as has been pointed out earlier in this discussion, the tendency is for either system to move toward an alkaline equilibrium state in the absence of carbohydrate, the greater the rate of acid production, the lower will be the pH value of the particular system. In other words, the pH difference between the control pH (the pH without glucose) and the actual pH (the pH with glucose), which will be referred to here as $-\Delta$pH (see subsection F above), will be a reflection of the rate of total acid production by the bacteria in each system.

Since enzymes act by forming intermediate complexes with the substrate, for any given enzyme concentration (or, in this case, enzyme units), as the substrate concentration increases, the over-all velocity of the reaction increases (in this case, process 1) until a maximum velocity, which is usually referred to as V_{max}, is reached. It must be remembered, however, that *the rate of change* of the velocity of the reaction decreases exponentially, reaching a value of zero at V_{max} (Fig. 10). This means that a "diphasic" activity curve will result, so that, at a low substrate concentration, the velocity of the reaction will depend upon (1) the inherent rate of the enzyme-catalyzed reaction or sequence of reactions and (2) the frequency of contact between enzyme (or enzyme sites) and substrate molecules, which in turn is a function of the substrate concentration.[21] On the other hand, at a substrate concentration greater than that required to saturate the enzyme or enzyme units (in this present study, at least above 5–10 per cent), the presence of excess molecules (in this case, glucose) will probably prevent the product formed from diffusing away from the cells and therefore produce an increase in the rate of process 2 and a slowing of process 1. This is seen in the present study in vivo with the 50 per cent glucose (Figs. 4, 9, *b*, and 10) and in vitro with concentrations of glucose above 5 per cent. In other words, at high substrate concentrations, the rate of process 1 is no longer dependent on the substrate concentration, as was the case at low glucose or sugar concentrations. However, if there is a net synthesis of further multienzyme units as will occur more readily in the saliva-glucose mixtures than in vivo and presumably more readily at certain substrate concentrations than at others, it then becomes possible for the rate of process 1 to proceed more rapidly than previously because of this increase. Therefore, the differences in the pH values observed in these mixtures can be expected to reflect the effect or effects of the glucose concentration on both bacterial growth and bacterial metabolism. This would appear to be in agreement with the findings of Stralfors[5] that bacteria metabolizing more rapidly also increase in number at a faster rate.

It should be pointed out here that in vivo, even though an optimal concentration of

glucose was supplied to the plaque bacteria, because the bacteria (being in high concentration) can produce acid rapidly, in a matter of minutes pH values in the plaque will fall to levels at which the rate of both metabolism and growth per cell are less, i.e., the rapid pH fall introduces a rapid counteracting force to any further growth. In vitro, however, because the bacteria are not "crowded," this retarding effect of low pH values does not come into play until the bacteria have increased in number. It is possible, therefore, that, in "crowded" bacterial populations as in plaque, low glucose or sugar concentrations (which will give a small pH drop and therefore pH levels that are more conducive to bacterial metabolism and growth) are more important for the growth of the acidogenic bacteria in a flora than the reverse situation, in which the glucose concentration is optimal and the pH is not.

Let us now proceed to consider the physical factors, namely diffusion, that might be expected to determine the amount of glucose available to the plaque bacteria for degradation to acid in the experiments carried out in vivo.

The in vivo system can be represented in the following way:

$$\begin{array}{ccccccc} & | & & | & & | & \\ & | & & | & & | & \\ & k_1 | & k_2 & | & k_3 & | & \\ G_s \rightarrow & | & G_p \rightleftarrows & A_p & | & \rightarrow & A_s \\ & | & k_{-2} & | & & | & \\ & | & & | & & | & \\ g_s & | & g_p & a_p & | & a_s & \\ & | & & | & & | & \end{array} \qquad (2)$$

where G_s is the glucose in the saliva, G_p the glucose in the plaque, A_p the acid in the plaque, A_s the acid in the saliva, and g_s, g_p, a_p, and a_s their respective concentrations, and k_1, k_2, k_{-2}, k_3 the appropriate rate constants. This expression is essentially the same as equation (1) given above, except that (1) because of the slower diffusion of glucose through plaque material than through saliva, the step from G_s to G_p becomes important in vivo but not in vitro, and (2) since experiments were carried out on the same plaques in each case, the concentration of multienzyme units can be considered to be approximately constant, the assumption being made from the previous discussion that there is little net synthesis of additional multienzyme units over the period in which these experiments were carried out. Lanke[11] found that in the 22 subjects she studied, the fasting salivary "sugar" concentrations were extremely low and varied from 0.002 to 0.006 per cent (although "sugar" concentrations would be higher if the polysaccharide component of saliva were included). However, with the introduction of glucose onto the plaque surfaces as in the present study, the above equilibrium for the fasting state was disturbed. When the glucose was added continuously, depending on the concentration added, a new equilibrium or steady state was reached. The explanation for these observations will become clearer after the rate equations for the above system are considered:[22]

$$\frac{dg_p}{dt} = k_1(g_s - g_p) + k_{-2}a_p - k_2 g_p, \qquad (3)$$

$$\frac{da_p}{dt} = k_2 g_p - k_{-2}a_p - k_3(a_p - a_s). \qquad (4)$$

For the steady state,

$$\frac{dg_p}{dt} = \frac{da_p}{dt} = 0,$$

and, by solving for g_p and a_p, the following values for g_p and a_p in the steady state are obtained:

$$g_p^S = \frac{(k_1 k_{-2} g_s + k_1 k_3 g_s + k_{-2} k_3 a_s)}{(k_1 k_{-2} + k_1 k_3 + k_2 k_3)}, \tag{5}$$

where g_p^S is the value of g_p in the steady state, and

$$a_p^S = \frac{(k_2 k_1 g_s + k_1 k_3 a_s + k_2 k_3 a_s)}{(k_1 k_{-2} + k_1 k_3 + k_2 k_3)}, \tag{6}$$

where a_p^S is the value of a_p in the steady state.

It is clear from equations (5) and (6) that the concentrations of G_p and A_p in the steady state are independent of the initial concentrations of G_p and A_p but are dependent on the rate constants and on the concentrations of G_s and A_s. It is also to be noted that the concentrations of G_p and A_p in the steady state are constant, although glucose is continuously entering the plaque and acid is diffusing out.

Since the small amount of acid diffusing out of the plaques that were studied above would not be expected to alter significantly the concentration of A_s, a_s can be considered to be approximately constant. From equation (6), therefore, it becomes clear that the concentration of A_p is a function of the concentration of G_s, the glucose in the saliva. In the present study this was clearly seen to be so in those experiments in which glucose was added continuously at different concentrations and the steady state was found to depend on the concentration of glucose added (i.e., g_s). It was also observed in these experiments that the cessation of the glucose additions resulted in a return toward the original state. Since the removal of the glucose by the saliva will cause the glucose concentration to fall, as predicted from equation (6), a_p will also fall, giving the pH rise that was observed. If, however, there is a rate-determining step, which in this case is the entry of the glucose into the cells and its conversion to acid (the limiting factor being the concentration of multienzyme units, each of which would be handling a maximum amount of glucose as pointed out above), increasing G_s can no longer lower the level of the steady state. With concentrations of G_s greater than that required to convert G_p to A_p at its maximum rate, G_p can be expected to accumulate, and the inhibition, as shown by the raising of the pH level, could be explained in the manner given earlier. The present observations provide evidence for the views of Widdas,[23] LeFevre and LeFevre,[24] and Fisher and Parsons[25] that the entry of glucose into cells cannot be explained by simple diffusion alone but can best be explained on the basis of "catalyzed diffusion,"[23] in which diffusion of glucose into the cell is determined by diffusion plus a saturation effect as above. The details of "catalyzed diffusion" (or "active transport") are under intensive study at the present time by numerous investigators but are not important to the present discussion.

Let us now consider what happens when the glucose additions are made for short intervals. The fasting glucose (or "sugar") levels in the saliva and in the plaque can be considered to be approximately constant. Since only a few seconds are required in

each case to make a single addition of glucose onto the surfaces of the plaques studied, the time required to make this addition can be considered to be approximately zero. This means that the glucose concentration at time zero on the plaque surface can be taken to be that concentration that has been added, allowing, of course, for any dilution that might have taken place. This added glucose will, according to the diffusion laws, tend to diffuse in all directions, therefore diffusing not only into the plaque but also away from the plaque surface into the saliva in the remainder of the mouth. Since glucose tends to diffuse more slowly through plaque material than through saliva,[17] the concentration of glucose in the plaque will tend to rise at first as the glucose on the plaque surface and in the saliva is falling, assuming for a moment that the glucose entering the plaque is not broken down by the bacteria. These points are illustrated in Figure 11, in which the solid lines represent the curves for the glucose concentrations in the plaque. Although the glucose concentration will presumably be different in different parts of the plaque,[5] for simplicity the plaque glucose concentrations will be taken here as the mean concentrations for the particular plaque being considered. The rate at which the glucose on the plaque surface (g_s) decreases will be a function of its concentration, assuming that the concentration of the glucose in the whole saliva remains unaltered by the small amounts of glucose added. This relationship can be expressed in the following way:

$$-\frac{d\,g_s}{dt} = k\,g_s,$$

where g_s is the glucose concentration in the saliva adjacent to the plaque surface, t is the time, and k is the rate constant. After integration, this becomes $g_s = g_0 e^{-kt}$, where g_0 is the concentration of g_s at time zero. This equation gives a family of curves (some of which are shown by the curves *A*, *B*, *C*, and *D*, in Fig. 11), g_0 representing the starting value and therefore the level at which the curve runs, while k determines the face and its concentration (cf. Rashevsky[26] for the single cell). The dotted line in the plaque surface falls, the glucose concentration in the plaque rises until the concentration gradient between them reaches zero. However, as the glucose concentration on the plaque surface continues to fall, the direction of the concentration gradient becomes reversed, and now the plaque glucose concentration falls along with that on the surface. Although in Figure 11 this portion of each of the curves is illustrated as decreasing coincidentally, it is probable that, because of the slower diffusion of glucose through plaque and its utilization by the plaque bacteria, these curves will be approximately parallel but not coincident. The reason for this will become clearer when Figure 12 is considered.

In Figure 12, *a*, the solid line gives the relationship expressed graphically that might be expected in the steady state between the distance of the glucose from the tooth surface and its concentration (cf. Rashevsky[26] for the single cell). The dotted line in the same figure gives the relationship that might be expected when the glucose has been added at time zero, and the relationships that might be expected until the original steady state in Figure 12, *a*, is again reached, are given in Figure 12, *b*, *c*, and *d*. The greater the initial glucose concentration, the greater will be the concentration gradient between the glucose on the plaque surface and that within the plaque. As a result, the plaque glucose concentration can be expected to rise more rapidly with these

higher concentrations. This is illustrated in Figure 11. This more rapid penetration with higher concentrations has been shown to occur by Muntz and Miller[17] in their study on the penetration of plaque material by various substances, chiefly detergents.

Since some concentration of glucose in the saliva on the plaque surface will give a maximum pH disturbance, to represent this concentration in Figure 11 a line is drawn parallel to the abscissa and is labeled G_s^{max}. It can be seen from this figure that if an addition of glucose of short duration, say a few seconds, is made onto the plaque surface, it must be of a much higher concentration than G_s^{max} if a concentration is to build up in the plaque to give a maximum pH response. If, however, the length of time that the glucose is applied to the plaque surface is increased to a few minutes (as in some of the experiments above), the glucose concentration now added does not have to be as high as previously to give a maximum pH disturbance, since the glucose has more time to diffuse into the plaque. In Figure 11 this type of glucose addition is given by the horizontal line *X* and the curve *D*, which has been extrapolated to zero time to

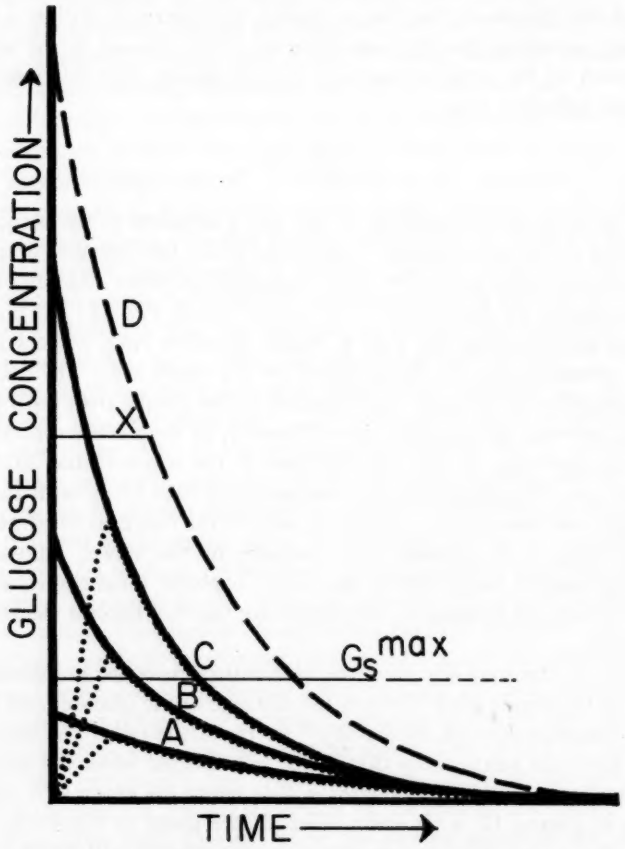

FIG. 11.—The effect of the concentration and length of time of addition of a glucose solution on plaque sugar levels. *A, B, C, D:* curves with different initial glucose concentrations; G_s^{max}: glucose concentration giving maximum pH decrease; *X:* addition of glucose for time *X*.

show what concentration of glucose added as a single addition of a few seconds' duration would be required to give a similar response.

It seems clear, therefore, that the longer the glucose is added, the lower the concentration need be to give a maximum response, provided that this concentration is above G_g^{max}. If this glucose is added continuously for a long enough time, the new steady state will be reached and be maintained as long as the plaque glucose level is at, or above, the level corresponding to G^{max}.

It might be added here that if the glucose level of the whole saliva is elevated, as with a glucose rinse (or with foods), in contrast to merely a localized portion of the saliva as in the experiments above, it would then seem that, because it requires a longer time to clear a larger quantity of glucose from the saliva than a smaller one, glucose would be available to the plaque bacteria for a longer time and therefore give a greater plaque pH disturbance. That is, the rate of fall of the glucose concentration on the plaque surface would be slowed and could, if Miller's Chemico-Parasitic Theory[27]

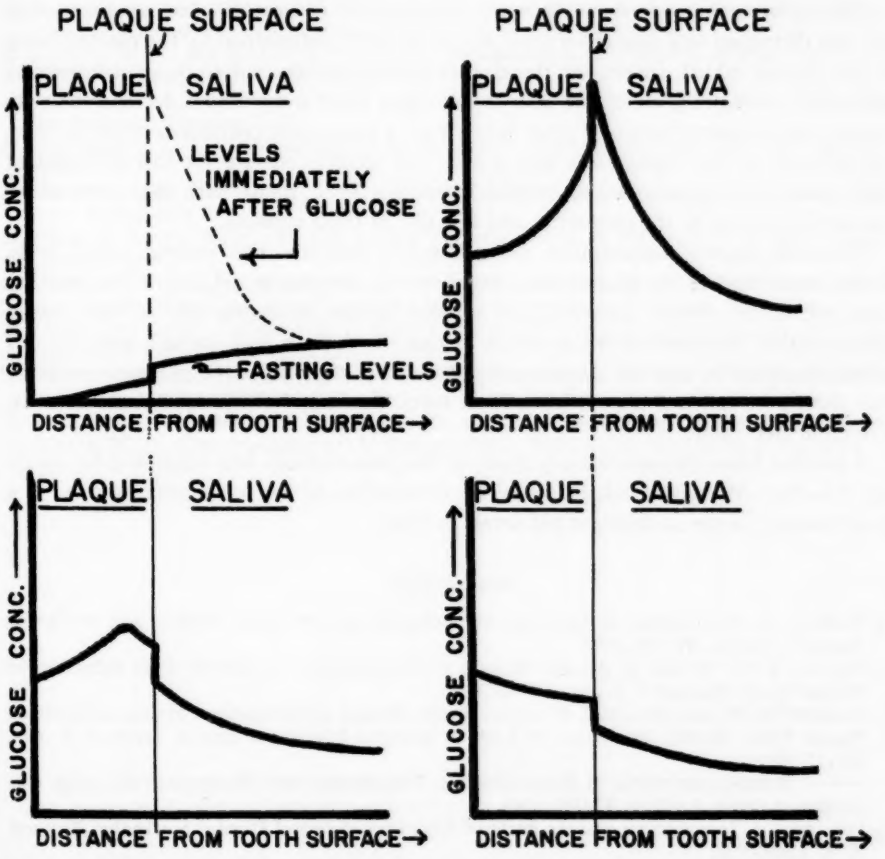

FIG. 12.—The relationship between the glucose concentration in the saliva and in the plaque and the distance from the tooth surface before and after an addition of glucose is made onto the plaque surface. (12, *a*, is upper left graph; *b*, upper right; *c*, lower left; and *d*, lower right.)

is correct, be extremely important in the caries process. The application of the above studies to the problem of the effects that carbohydrate foodstuffs might have on plaque pH levels and presumably on the caries process has been considered elsewhere.[12, 14, 15]

SUMMARY

The effect of different concentrations of glucose on the pH obtained in saliva-glucose mixtures on incubation for 24 hours has been studied. With increasing concentrations of glucose, a greater pH fall was produced until a maximum was reached at approximately 5 per cent, but, with progressively higher glucose concentrations, this pH fall became progressively less.

By the local application of glucose to selected plaque areas *in situ,* the effects of different glucose concentrations under various conditions on the pH of these plaques were studied. Under these conditions, the results clearly pointed to a cause-and-effect relationship between the presence of glucose and the pH fall in these plaques; i.e., adding glucose caused a pH fall, while removing the added glucose gave a pH rise.

When glucose was added continuously over a period of time, the fasting steady-state pH was disturbed to a new lower level, this level being determined by the concentration of the glucose added. Increasing the glucose concentration resulted in a corresponding decrease in the pH level of the new steady state until a maximum decrease was obtained with a concentration of 5 per cent. With a glucose concentration of 50 per cent, the pH level of the steady state was raised. The possible reasons for this inhibition at high glucose concentrations, as in vitro, were discussed, along with the above observations in relation to the properties and kinetics of these systems.

When the glucose concentration was allowed to decrease from various initial levels, it was found that as the glucose concentration fell, the plaque pH also fell at first but then rose as the glucose concentration fell still further. If during this fall the glucose concentration remained above a certain critical level for a long enough time, the pH minimum could be reached. It was pointed out that the longer the glucose concentration during its fall was above this critical level, the longer the plaque could be at its minimum pH value.

A method based on observations made in the present study was suggested for studying the effect of a given substance on the metabolism of the acidogenic bacteria in a given dental plaque at different pH levels in vivo.

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The Principle of Bacterial Antagonism Applied as an Aid in the Reduction of Dental Caries

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The oral flora consists of a variety of micro-organisms both pathogenic and non-pathogenic.[1, 2] Some of the organisms are antagonistic to the growth of pathogens and therefore may be called protective.[3–5]

Many oral organisms, particularly the acidogenic varieties, have been implicated in dental caries.[6–10] In vitro and clinical studies show that the growth of lactobacilli and other oral acidogenic organisms has been inhibited by certain indigenous strains.[11–15] This inhibition may be a beneficial factor in reducing the incidence of dental decay. It occurred to us that the establishment in the mouth of an exogenous, non-pathogenic organism shown capable by in vitro studies of inhibiting the growth of acidogenic organisms might reduce dental caries activity. This paper reports the result of a clinical study in which a strain of *Bacillus brevis* was used as an oral inoculum.

EXPERIMENTAL METHODS

Preliminary laboratory tests indicated the existence of many possible antagonists.[16] *Bacillus brevis* proved to possess properties most suitable for our experiment.[5, 17] Test animals, caries-susceptible hamsters, were given daily oral inoculations from birth with a salivary-adapted strain of *B. brevis*. No preliminary determinations were made to establish the presence or absence of *B. brevis* before inoculation. The experimental and control animals were placed on a caries-producing diet for 100 days. Dental caries was reduced 50 per cent in the inoculated animals when compared with a control group.[18] The method described by Keyes[19] was used to score the caries incidence. Toxicity tests proved the organisms to be non-toxic.[17]

Encouraged by these findings, which indicated the caries-preventive properties of this bacterial antagonist, we initiated a clinical investigation.

Number 1.—The subjects for this study were chosen from the student body and general clinic patients at the college. All subjects were examined clinically and roentgenographically on two or more occasions. The age, sex, number of teeth present, the DMFT-deft rate, and the number of months intervening between examinations were recorded.

"Number of teeth present" included all teeth present that were sufficiently erupted to expose 1 mm. of occlusal height regardless of their condition.

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"DMFT-deft" was indicated by the number of teeth having active caries and/or restorations, and those teeth missing from the normal dentition with the exception of restorations which were regarded as having been placed for aesthetic or abutment purposes. Teeth designated as "missing" included only those that evidence indicated had been lost through caries.

Fifty-four persons comprised the experimental group, and 126 constituted the control group.

Number 2.—The control group received an antibiotic mouth rinse composed of Bacitracin 10 mg., Dihydrostreptomycin 10 mg., and Mycostatin 10 mg. in 1 liter of distilled water at the time of each examination. They received no other treatment.

Number 3.—The experimental group received the following treatment. Before each application of *B. brevis,* the antibiotic mouthwash of Bacitracin, Dihydrostreptomycin, and Mycostatin was employed to reduce the established oral flora and prepare the field for the introduction of the antagonist. Inoculation of *B. brevis* was achieved

TABLE 1

AGE DISTRIBUTION OF SUBJECTS IN EXPERIMENTAL AND CONTROL GROUPS

Age Group (Years)	Experimental		Control	
	No. of Persons	Approx. Per Cent	No. of Persons	Approx. Per Cent
6–12	10	22	22	22
13–19	7	14	14	14
20–35	19	43	44	43
36–49	8	17	17	17
50 and over	2	4	4	4
Total	46	.....	101	.....

either by application of the vegetative forms in a saline suspension directly to the teeth or by dry spores suspended in diatomaceous earth insufflated upon the surfaces of the teeth. Patients were inoculated approximately every 90 days.

Bacterial cultures were taken from the oral cavity at the time of the second examination. Early results showed that the implanted organisms seldom remained in the mouth for more than a few hours. With improved techniques using organisms adapted to thrive in the oral environment[17] and by improving the methods of inoculation and recovery, seven persons retained the organism in the oral cavity more than 30 days, two for as long as 7 months. We now expect every inoculated person to retain the organisms for an average of 30 days.

More than 400 persons have received *B. brevis* inoculations in this and in related projects. No toxic effects have been noted over periods of time up to 15 years.[13]

RESULTS

In stratifying the subjects into comparable age groups for statistical analysis, the number of subjects was reduced to 46 in the experimental group and to 101 in the control group. In the experimental and control groups the age distribution was as is shown in Table 1.

The figures in Table 2 indicate that the average number of new DMFT-deft per subject in the experimental group was 50 per cent less than that of the control group. This difference was significant at the 5 per cent confidence level.

A comparison of the percentage of patients with no DMFT or deft increment was made between the two groups. Seventy-four per cent of the patients in the experimental group showed no new DMFT-deft. Only 50 per cent of the patients in the control group showed no new DMFG-deft (see Table 2). The chi-square test indicated that this difference was statistically significant at the 1 per cent level.

TABLE 2

CARIES EXPERIENCE IN EXPERIMENTAL AND CONTROL GROUPS

	Experimental Group	Control Group
Number of patients	46	101
Average age (years)	25.6	25.8
Sex distribution (per cent)		
Male	54	34
Female	46	66
Average number of months between first and last examinations	16.4	16.4
Average number of teeth at first examination	26.3	24.9
Average number of DMFT-deft at first examination	18.1	18.4
New DMFT-deft: total for group	34	150.0
Average number of new DMFT-deft per subject	0.74	1.48
Patients showing no new DMFT-deft	34 (74%)	50 (50%)

DISCUSSION

Further study of the action of bacterial antagonists in the reduction of dental caries is at present following two programs of investigation. First, a number of strains of organisms showing antagonistic properties are being studied to reveal, if possible, a strain or combination of strains that will prove more effective than *B. brevis* alone. Second, an additional study incorporating a much larger group of subjects is already under way. Essentially the same procedures are being employed as in the survey just completed.

SUMMARY

A strain of *B. brevis* with known in vitro inhibition to the growth of oral acidogenic micro-organisms was introduced into the mouths of 46 human volunteers. A group of 101 persons served as controls. The incidence of dental caries was reduced by 50 per cent in the experimental group.

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Micro-indentation Hardness

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Hardness testing has found wide application as a method for the classification of materials and for the comparative study of changes in their properties. Hardness is usually determined by abrasion tests, by scratch tests, or by indentation testing.[1] The latter method, however, has found most extensive application.

The Brinell test, first described in 1900,[2] is conducted by pressing a steel ball into the surface to be tested until a specified load is reached. The Brinell hardness, H_B, is computed as the ratio of the load (3,000 kg.) to the contact area in square millimeters.

The Rockwell hardness testing method is essentially based on a similar principle. The range of application of Brinell and Rockwell testing is limited by the fact that steel-ball indenters may be deformed when used on very hard materials.

Smith and Sandland[3] used polished diamond pyramids with angles of 120°–150° and, as a result of practical and theoretical considerations, recommended the use of square-based diamond indenters with the 136° included angle, commonly known as the Vicker's diamond indenter. The Vicker's hardness is normally determined by using loads varying from 1 to 120 kg. The geometry of the Vicker type indentation is independent of the penetration, and thus it would be expected that the hardness would be independent of the load. This relationship does not prevail with the Brinell method. The angle which the indentation makes with the surface of the test piece changes with the depth of penetration.

For a given ball diameter, Meyer[4] has established the relationship: $L = a\ d^n$, where L = load, a and n are constants for a given material, and d is the diameter of the indentation. When the exponent, n, equals 2, there is proportionality between the load and the area of the indentation—in other words, the hardness is load-independent. This is also expressed by the equation $H = b\ d^{n-2}$, when b and n are the material constants.

Determination of the exponent, n, from the relation $L = a\ d^n$ has thus been used as a test for hardness-load dependence under the name of "Meyer Analysis." If n is larger than 2, the hardness increases with increasing loads, whereas n-values less than 2 indicate a decrease in hardness with increasing loads. Meyer analysis has been carried out by several investigators,[5–8] both with Brinell and Vicker's hardness determinations at different loads.

Determination of the so-called micro-hardness or, to use a more concise term, the

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micro-indentation hardness, is carried out at low loads. Micro-indentation hardness testing equipment, frequently used in dentistry, includes the Kentron* hardness tester and the Tukon† hardness tester, both of which are used with diamond indenters. Two types of indenters are commonly used with both testing machines, namely, Vicker's diamond indenter of 136° and the Knoop diamond indenter of 172½° included angle. A spring-loaded micro-hardness instrument for Knoop indentations with loads between 40 and 600 mg. was described by Richardson and Worner[9] in 1945.

Dental applications of micro-indentation hardness determinations were initiated by Hodge,[10] who reported hardness figures for dentin. Knoop, Peters, and Emerson,[11] in 1939, described the Knoop indenter and reported hardness values for many materials, including gypsum, natural apatites, and several metals. These authors also mentioned that the indenter had been used for testing glasses, dental plastics, enamels, and porcelains. Richardson and Worner[9] published data on similar materials.

Souder and Schoonover,[12] in 1943, reported hardness values for enamel, and Gustafson and Kling,[13] in 1948, also used micro-indentation hardness determinations in their studies of enamel. Swartz and Phillips,[14] Atkinson and Saunsbury,[15] Caldwell, Muntz, Gilmore, and Pigman,[16] Craig and Peyton,[17] Newbrun, Timberlake, and Pigman,[18] all employed this type of testing in evaluation of enamel hardness.

A review of the testing methods used in the afore-mentioned studies reveals that considerable variations exist in the loads used, the rates of load application, dwell times, the data interpretation, and the specimen preparation.

Considerable research has been reported on the load dependence of Vicker's micro-indentation hardness.[5–8, 11] Vitovec and Binder[19] employed hardness determinations and indicated discontinuities in the slope of the hardness-load curves for several metals. These authors pointed out that strain hardening due to specimen preparation may influence the hardness-load relationship.

Limited data are available on hardness-load dependence for the Knoop method. Since this type of micro-indentation hardness determination is gaining widespread use in dentistry, it was decided to conduct a study of the hardness-load dependence for the Knoop hardness of several materials. For comparison, a similar analysis was also conducted for Vicker's hardness.

MATERIALS AND METHODS

In the present study, Knoop and Vicker's type diamond indenters were used in a micro-hardness tester‡ to determine the hardness values at loads of 1, 5, 10, 25, 50, 100, 1,000, and 10,000 gm. for the materials listed in Table 1. The hardness tester was mounted on rubber shoes on a table which rested on soft rubber pads at the base of the legs to prevent external vibrations. Furthermore, care was taken to prevent contact with the table and the tester during the testing cycle.

Specimen treatment.—The specimens were mounted in self-curing resin and polished with aloxite-paper, Nos. 0, 2/0, 3/0, and 4/0. Final preparation was done with

* Christian Becker Co., Clifton, N.J.

† Wilson Mechanical Instruments Division, American Chain and Cable Co., Inc., New York City.

‡ Kentron.

TABLE 1

RESULTS OF KNOOP AND VICKER'S HARDNESS DETERMINATIONS OF 13 MATERIALS USING LOADS FROM 1 TO 10,000 GM.

Material	Load (Gm.)	Knoop Indenter				Vicker's Indenter			
		M Mean of Filar Unit Readings	Coeff. of Variation in Per Cent of *M*	H_K Knoop Hardness	Revised *n*-Values	*M* Mean of Filar Unit Readings	Coeff. of Variation in Per Cent of *M*	H_V Vicker's Hardness	Revised *n*-Values
Methyl methacrylate	1	*	.....	.....	*	*	.....	.....	*
	5	284	4.4	20	1.94	*	.....	.....	*
	10	414	1.3	19	1.95	160	1.9	14	2.05
	25	*265*	0.3	19	1.96	252	1.0	15	2.02
	50	*380*	1.1	19	1.94	*146*	2.0	17	2.02
	100	*550*	0.5	18	1.96	*203*	0.6	17	2.02
	500	**590**	0.6	17	1.92	*452*	0.8	17	2.01
	1,000	**849**	0.3	17	1.95	**309**	0.6	17	2.02
	10,000	*	.....	.....	*	**957**	0.7	17	2.05
Silicate cement	1	112	2.2	26	2.37	*	.....	.....	*
	5	172	4.9	56	2.19	*	.....	.....	*
	10	241	5.1	52	2.21	85	10.8	58	2.19
	25	*157*	3.0	56	2.26	134	6.2	60	2.21
	50	*210*	4.9	63	2.24	187	7.2	61	2.22
	100	*286*	5.3	68	2.24	255	3.9	66	2.22
	500	*616*	2.6	78	2.23	*208*	1.7	83	2.19
	1,000	**399**	2.2	78	2.36	*281*	0.5	89	2.17
	10,000	*	.....	.....	*	**391**	0.5	105	2.21
Dental amalgam	1	61	8.7	84	2.03	*	.....	.....	*
	5	137	10.4	89	2.02	55	2.5	41	2.06
	10	182	8.5	100	1.99	66	8.2	99	1.98
	25	279	17.8	106	1.97	102	5.3	103	1.97
	50	397	5.9	105	1.97	139	2.7	111	1.94
	100	561	8.0	105	1.96	207	3.2	100	1.97
	500	*528*	4.9	99	1.98	*193*	2.8	96	1.98
	1,000	*807*	1.8	82	2.15	*278*	3.0	92	1.99
	10,000	*	.....	.....	*	**440**	3.1	83	2.11
Cast copper	1	80	6.6	51	2.26	42	5.0	24	2.41
	5	133	5.7	93	2.13	64	1.7	52	2.27
	10	185	7.9	97	2.13	74	2.8	76	2.18
	25	272	1.7	112	2.10	102	2.8	103	2.13
	50	382	1.8	113	2.10	145	2.4	102	2.14
	100	493	1.4	135	2.03	199	1.6	108	2.14
	500	*520*	0.7	101	2.26	*184*	0.4	105	2.20
	1,000	*729*	0.8	103	2.36	*263*	0.9	102	2.25
	10,000	*	.....	.....	*	**410**	0.2	95	2.69
Dentin	1	104	7.4	30	2.32	*	.....	.....	*
	5	170	5.2	58	2.18	*	.....	.....	*
	10	221	3.6	68	2.14	78	6.9	71	2.01
	25	352	4.8	61	2.18	121	5.9	73	2.00
	50	*195*	3.7	72	2.17	182	1.2	65	2.04
	100	*281*	1.7	69	2.22	231	3.8	80	1.97
	500	*620*	1.7	71	2.34	*227*	3.3	69	2.02
	1,000	*819*	0.8	82	2.29	*318*	4.6	71	2.02
	10,000	*	.....	.....	*	*980*	1.6	74	1.97

* Acceptable indentations were not recorded for this load.
Lens Factor No. 1, 0.2083: roman type.
Lens Factor No. 2, 0.511: italic type.
Lens Factor No. 3, 1.075: boldface type.

TABLE 1—*Continued*

Material	Load (Gm.)	Knoop Indenter				Vicker's Indenter			
		M Mean of Filar Unit Readings	Coeff. of Variation in Per Cent of *M*	H_K Knoop Hardness	Revised *n*-Values	*M* Mean of Filar Unit Readings	Coeff. of Variation in Per Cent of *M*	H_V Vicker's Hardness	Revised *n*-Values
Dental enamel	1	52	5.0	123	2.28	*	.....	.....	*
	5	72	3.7	319	2.08	*	.....	.....	*
	10	104	8.6	308	2.09	42	3.6	242	2.04
	25	159	7.9	327	2.09	63	4.8	270	2.02
	50	219	1.0	345	2.08	82	4.6	303	1.98
	100	305	2.6	355	2.08	111	1.8	348	1.95
	500	*301*	2.6	303	2.20	251	2.7	339	1.94
	1,000	*436*	2.9	289	2.28	358	0.6	335	1.95
	10,000	†	.....	.....	*	*539*	2.3	254	2.14
Gold foil	1	102	8.3	32	2.35	*	.....	.....	*
	5	156	3.0	68	2.16	*	.....	.....	*
	10	219	8.1	69	2.18	88	3.9	55	2.10
	25	312	5.0	85	2.14	121	5.3	73	2.03
	50	*179*	4.5	86	2.14	170	2.0	74	2.04
	100	*264*	5.0	74	2.21	235	2.3	77	2.02
	500	*583*	1.6	81	2.32	*215*	1.4	77	2.03
	1,000	**377**	1.0	87	2.35	*308*	1.4	75	2.05
	10,000	*	.....	.....	*	**463**	0.9	75	2.14
Gold band 22K	1	85	5.3	45	2.12	49	3.3	18	2.27
	5	138	6.0	87	1.96	72	10.8	45	2.09
	10	212	7.5	74	2.01	86	4.8	58	2.01
	25	279	3.7	106	1.88	119	6.0	70	1.93
	50	421	1.1	94	1.91	164	1.3	80	1.91
	100	622	1.5	85	1.94	236	2.0	75	1.91
	500	*628*	2.0	70	2.07	*232*	5.4	61	1.95
	1,000	**456**	1.2	60	2.34	*341*	2.5	67	1.98
	10,000	*	.....	.....	*	†	.....	.....	.....
Gold alloy, type B	1	53	14.5	119	2.27	29	6.8	51	2.52
	5	85	6.0	227	2.14	43	5.8	117	2.38
	10	115	4.6	249	2.13	47	7.0	194	2.28
	25	166	6.8	293	2.09	61	6.5	287	2.20
	50	226	4.4	325	2.07	82	3.9	320	2.20
	100	323	3.2	320	2.09	114	2.4	330	2.21
	500	718	1.8	323	2.12	*106*	1.0	318	2.28
	1,000	*425*	0.4	305	2.18	*152*	2.5	308	2.32
	10,000	**635**	0.3	308	2.70	**235**	1.7	317	2.64
Gold alloy, type D	1	52	4.2	123	2.20	32	4.3	42	2.52
	5	97	4.1	176	2.14	45	6.0	105	2.35
	10	129	3.8	199	2.12	50	6.4	95	2.25
	25	199	3.5	208	2.11	70	5.5	220	2.21
	50	252	3.4	260	2.05	91	4.2	259	2.18
	100	345	1.6	277	2.04	*52*	1.9	264	2.19
	500	814	1.1	248	2.11	*120*	1.2	247	2.27
	1,000	*474*	0.5	243	2.16	*172*	1.1	240	2.32
	10,000	**718**	0.8	241	2.90	*545*	0.8	240	2.62
Steel testing block Rockwell C-25.5	1	*	.....	.....	*	*	.....	.....	*
	5	91	9.4	201	2.10	*	.....	.....	*
	10	119	9.5	233	2.07	43	5.8	231	2.03
	25	164	2.9	307	1.99	63	6.7	270	2.00
	50	232	3.0	308	1.99	101	1.1	210	2.06
	100	328	1.8	308	1.99	128	1.3	260	2.00
	500	777	1.6	274	2.05	290	1.6	254	2.01
	1,000	*441*	0.8	284	2.04	408	1.0	257	2.01
	10,000	**678**	0.2	270	2.32	*528*	0.3	254	2.03

† The specimen ruptured during the testing procedure.

TABLE 1—*Continued*

Material	Load (Gm.)	Knoop Indenter				Vicker's Indenter			
		M Mean of Filar Unit Readings	Coeff. of Variation in Per Cent of *M*	H_K Knoop Hardness	Revised *n*-Values	*M* Mean of Filar Unit Readings	Coeff. of Variation in Per Cent of *M*	H_V Vicker's Hardness	Revised *n*-Values
Ticonium	1	30	14.0	374	2.04	*	.....	.....	
	5	70	11.8	340	2.04	*	.....	.....	*
	10	94	7.7	373	2.01	40	2.5	267	2.07
	25	133	7.1	468	1.95	56	2.3	337	2.02
	50	187	4.8	475	1.94	70	4.1	435	1.97
	100	*108*	2.3	471	1.94	104	4.7	394	1.99
	500	*259*	4.7	412	1.98	245	1.0	356	2.02
	1,000	*393*	3.5	355	2.06	345	2.4	355	2.02
	10,000	**594**	0.9	352	2.26	**216**	1.1	345	2.05
Steel testing block Rockwell C-63.5	1	*	.....	.....	*	*	.....	.....	*
	5	*	.....	.....	*	*	.....	.....	*
	10	70	11.0	684	2.03	30	4.3	475	2.17
	25	93	6.8	954	1.95	38	7.6	725	2.09
	50	127	3.9	1023	1.92	52	4.6	795	2.08
	100	189	2.9	925	1.94	69	6.8	894	2.06
	500	446	2.8	832	1.97	158	0.8	855	2.08
	1,000	643	1.3	800	1.98	227	1.5	829	2.10
	10,000	**403**	0.7	766	2.01	*294*	0.3	824	2.16

1550AB No. 1 polishing alumina on a low-speed metallurgical polisher at very light load on the specimen.

Testing procedure.—The specimens were mounted on the stage of the testing machine in a horizontal position. Indentations were made with a rate of descent of 20 seconds for the complete operating lever traverse and a dwell time of an additional 20 seconds. Five acceptable indentations were made with each of the loads previously listed. The indentations were never closer to any edge of the specimens or to another indentation than two to three times the length of the diagonal. Criteria for accepting an indentation were sharpness of diagonal edges, uniformity of diagonal shape (geometry) and freedom of interference from irregularities in the testing area.

RESULTS

The arithmetic mean of five readings of acceptable indentations in filar units, the coefficients of variation in per cent, and the Knoop and Vicker's hardness values obtained on the basis of the filar readings are listed in Table 1 for each material and each load.

It should be noted that the coefficients of variation in per cent are computed on the basis of the readings of filar units which constitute the observed data. Mean filar unit readings are transposed into lengths of diagonals by multiplication with a lens factor, and the hardness values are then obtained from tables by multiplication of tabulated values by the applied loads. As a result of the various factors entering the computation, the coefficients of variation in per cent computed on the basis of the observed data are more meaningful than a coefficient of variation in per cent determined

on the basis of hardness values. Furthermore, the computation of hardness values on the basis of mean values of the five readings is not quite as laborious as the computation of a hardness value for each indentation. Such computations were, however, carried out for the highest and the lowest readings for each lens factor, in order to establish the relationship between coefficients of variation in per cent for filar units and for hardness values. Table 2 shows this relationship.

Figure 1 shows, for one of the materials tested, a graphical representation of the load-hardness relationship, with standard deviations indicated. It should be noted that the standard deviation decreases with increasing load for each lens factor.

The material constant, a, in the equation $L = a\ d^n$, or $\log L = \log a + n \log d$ was determined graphically, by plotting load value, L, in kg., as a function of the diagonal, d, in mm., on log-log paper. The value, a, for each material was read as the ordinate where the resulting curve crossed the vertical line: $d = 1$ mm. The exponent, n, was

TABLE 2

RELATIONSHIP BETWEEN COEFFICIENTS OF VARIATION IN PER CENT FOR FILAR READINGS AND FOR HARDNESS NUMBERS

Lens Factor	Filar Reading	Coefficient of Variation in Per Cent	Mean Knoop Number	Coefficient of Variation in Per Cent
0.2083.....	718	1.8	322	3.8
	30	14.0	325	13.0
0.511.....	849	0.3	17	0.0
	377	1.0	87	3.2
1.075.....	819	0.8	82	1.5
	157	3.0	56	6.2

determined from the values of L, d, and a, from the relation: $n = (\log L - \log a)/\log d$. Next, the Knoop and Vicker's hardness values and the exponent, n, were plotted as a function of load. The graphs shown in Figures 2 and 3 depict the results of the Meyer analysis described above for the thirteen materials tested.

Since the n-values obtained appeared to be, in general, slightly lower than one would expect from theoretical considerations (see the discussion below), the value, a, for each material was also computed by the analytical method (least squares).* The new a-values were then used to compute revised n-values for each material for each load. These are the n-values listed in Table 1 for both indenters.

DISCUSSION

The hardness values in Table 1 and in Figures 1–3 demonstrate that hardness is load-dependent for all the materials tested and particularly at low loads. As the load is increased, the hardness value increases until a maximum is reached, usually for a load of 50 or 100 gm. At loads of 500, 1,000, and 10,000 gm., the hardness values tend to decrease slightly, although they remain in the same order of magnitude as the maximal values.

* An IBM 650 Computer was used.

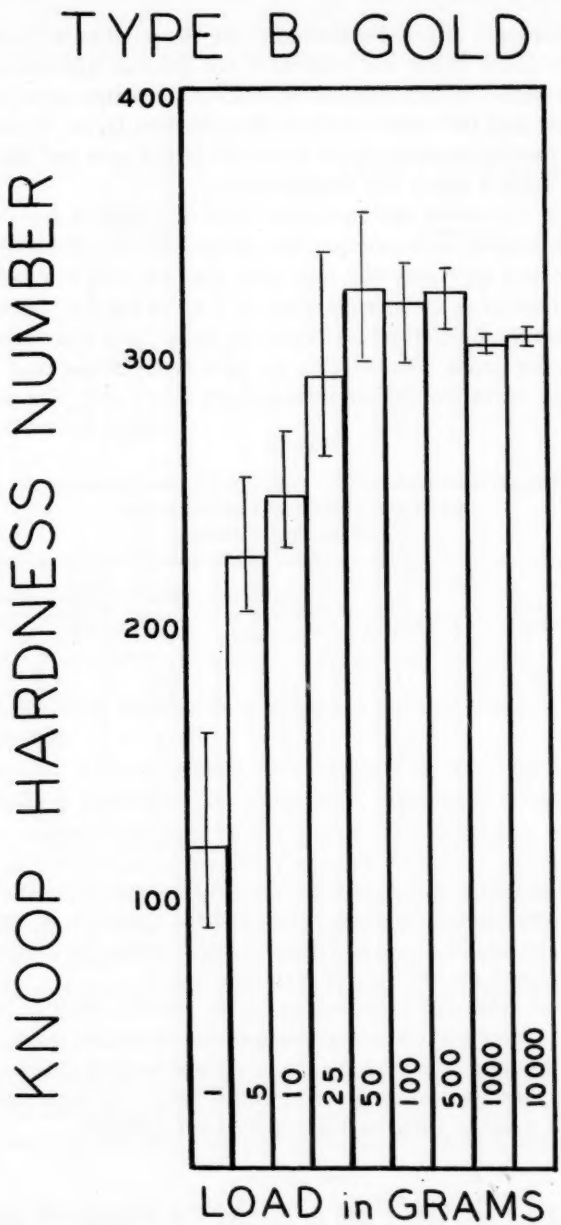

FIG. 1.—Graphical representation of the relationship between load in gm. and Knoop hardness number for type B dental casting gold. The standard deviations are indicated by the thin vertical lines at the top of the bars.

The coefficients of variation decrease with increase in load. Vitovec and Binder[19] found an initial increase in hardness with increasing load. They attributed this to "strain hardening and the effect of the free surface on the local yield strength." They also found that the exponent, n, in the relationship $L = a\,d^n$ reaches a maximum value between loads of 5 and 10 gm. The present study does not confirm this latter finding; however, the hardness values in Table 1 and the graphical illustrations of the hard-

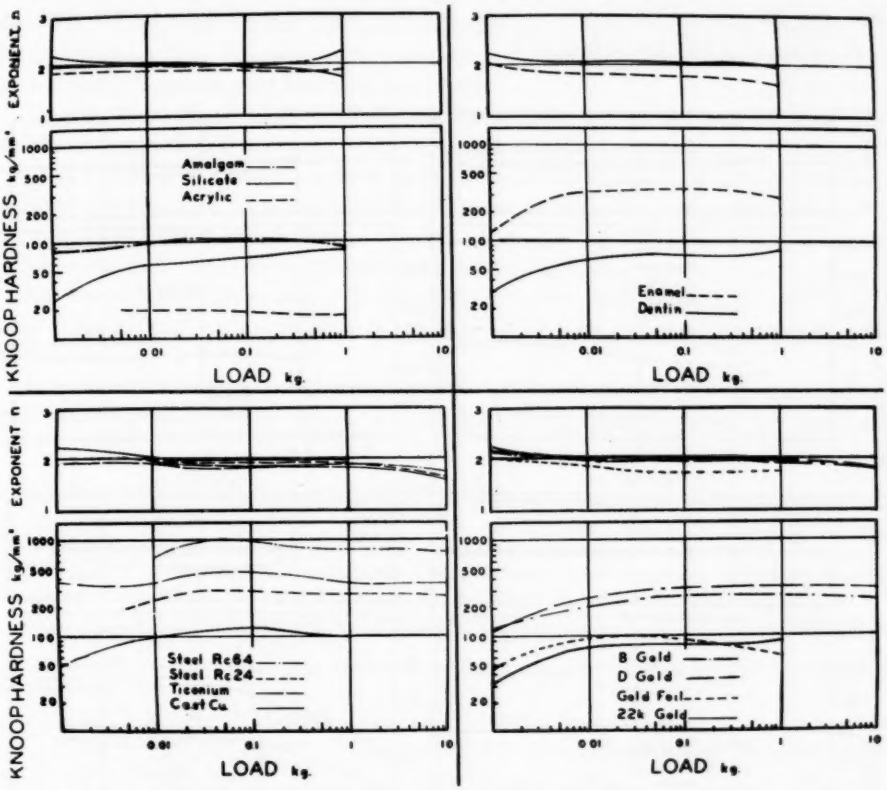

FIG. 2.—Knoop hardness and exponent n plotted as functions of load in kg. for the 13 materials tested (see text).

ness-load dependence are in general agreement with the results obtained by these authors except for low loads (25 gm. or less).

A comparison of Knoop and Vicker's hardness values for each material shows that approximately the same hardness value is obtained by either indenter, provided that the load is at the level of 50 or 100 gm. or over. Only the very hard materials (Rockwell testing blocks and Ticonium*) differ in this respect. Agreement between Vicker's and Knoop hardness requires loads of 1,000 gm. or over for these hard materials. In the low-load range (25 gm. or lower), the load-hardness dependence was most pro-

* CMP Industries, Inc., Albany, N.Y.

nounced for the Vicker's indenter. Also, in many instances the low-load indentations with the Vicker's indenter were not acceptable.

When the materials constants, a, were determined graphically, the resulting n-values represented in Figures 2 and 3 were in many instances lower than the values expected on the basis of theoretical considerations of the equation $L = a\ d^n$ and lower than values previously reported in the literature. In the graphical determination of the a-values the *straight portions* of the curves of load versus indentation length were projected to the vertical line at $d = 1$ mm., and this procedure accounts for the resulting high values of a and low values of n.

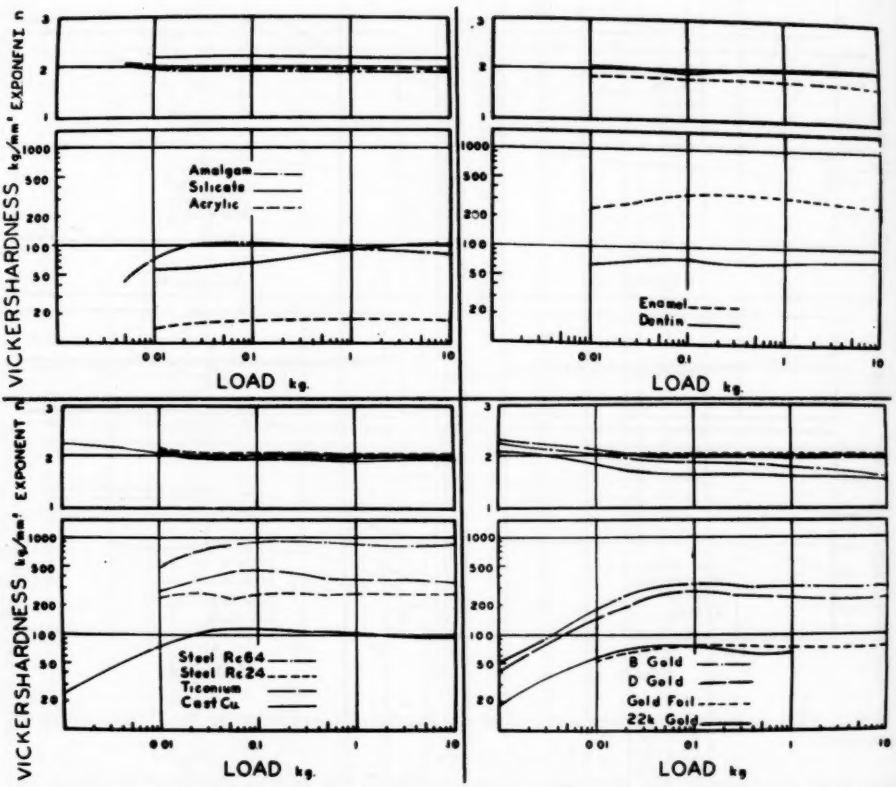

FIG. 3.—Vicker's hardness and exponent n as functions of load in kg. for the 13 materials tested (see text).

In the analytical method the projection of the curves computed by the least-squares scheme resulted in lower a-values throughout, since the low-load values of the indentation lengths were given equal consideration in the computations. The resulting sets of n-values were, in general, higher than those obtained from the graphically determined a-values, and they still illustrate the existence of load dependence. In most materials tested, the revised n-values changed from a higher value at the lower end of the load spectrum to a minimum for loads of 50–100 or 500 gm., after which some increase in n

was seen. This observation again suggests that hardness testing in the very low-load range (25 gm. or lower) is undesirable for most materials used in dentistry. In this study the n-values for low loads (25 gm. or less) did not follow the pattern described by Vitovec, regardless of the method of determination of the materials constant, a.

SUMMARY

Knoop and Vicker's micro-indentation hardness values for loads of 1, 5, 10, 25, 50, 100, 500, 1,000, and 10,000 gm. were reported for human enamel and dentin, for methyl methacrylate, silicate cement, amalgam, gold foil, gold band, two casting golds, cast copper, Ticonium, and two steel test blocks.

The results of Meyer analysis of the tests were presented in the form of tables and graphs of hardness and exponent, n, as functions of load.

Both Knoop and Vicker's hardness increased with increasing load until a maximum value was reached for a load of 50–100 or 500 gm. A slight decrease in hardness value resulted from further load increase.

Reproducibility of results improved with increasing loads.

For the materials here tested, the exponent, n, in the relationship $L = a\ d^n$ did not exhibit the type of variations at very low loads (5–10 gm.) claimed by other investigators.

It is advocated that the test loads be reported together with Knoop and Vicker's micro-indentation hardness values. This is particularly desirable when low loads are used.

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Fate of Subcutaneous Anorganic Bone Implants

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Anorganic bone is prepared from bovine bone by chelation with ethylene diamine to remove the organic matrix.[1] The remaining mineral is maintained in the original bone organ form. The cortex and cancellous portions may easily be cut with a sharp knife into any desired shape; also, it may be prepared into smaller granules of any desired mesh.

Anorganic bone, either cancellous or cortical, in large pieces or granules, has been implanted in intra-bony sites both in experimental animals and in humans. It was reported that bone tissue accepted favorably implants of anorganic bone.[2] Histologic studies have shown that anorganic bone serves as a nidus for bone apposition. Inferences have been made of the osteoblast-inducing properties of anorganic bone.[3] In addition, anorganic bone has been reported to be removed by osteoclastic resorption.[2] In one study, anorganic bone was implanted in the soft tissue of the abdominal wall in dogs. After 6 months the implant disappeared—presumably, it had been resorbed.[4]

The fate of the implants of anorganic bone is not entirely clear. There is the question of the inherent ability of anorganic bone to induce osteoblast production in connective tissue. For example, can anorganic bone induce the production of osteoblasts in the subcutaneous connective tissue? Another question concerns the osteoclastic resorption of anorganic bone. Do osteoclasts resorb bone free of organic matrix? It is generally believed that osteoclasts remove the organic matrix.[5] The mineral element is liberated into the tissue fluid. Moreover, can anorganic bone induce osteoclast production in non-bony connective tissue?

Histologic studies of anorganic bone implants were done with decalcified specimens. As anorganic bone is essentially mineral, the fate of this component is not known. Implants should be studied in the undecalcified state.

The fact that anorganic bone retains its bone organ form following chelation suggests two questions: Is the mineral component bound only by molecular crystalline continuity? Is there a residual mucopolysaccharide matrix binding the mineral component of anorganic bone?

To answer some of the above questions regarding the fate of anorganic bone, subcutaneous implants were studied in the undecalcified state.

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MATERIALS AND METHODS

Forty-mesh, sterile, anorganic bone* was implanted subcutaneously in the interscapular area of forty male albino mice. Under sterile conditions, a small, 1-cm. incision was made; the loose tissue was elevated and the bone implanted about 1 cm. distal to the line of incision. Boiled beef-bone spicules and spicules of fresh isologous rib bone, approximately 40 mesh, implanted subcutaneously by a similar procedure served as local controls. The incision was closed by interrupted 000 silk sutures. Four mice were sacrificed at intervals as follows: 24, 36, 72 hours; 1 week; 1 month; 2, 3, 4, 5, and 6 months.

The skin of the implant site was excised in-block and fixed in 10 per cent formalin. The tissue was subsequently imbedded in paraffin and sectioned at 6 μ. The sections were stained for phosphate and carbonates of calcium by the method of von Kossa; for mucopolysaccharide by the P.A.S. reaction modified by Coleman; and for routine examination by hematoxylin and eosin.

RESULTS

Connective-tissue reaction.—The subcutaneous tissue reactions to the implants of anorganic bone, heterologous bone, and isologous bone were similar. At 3 days, a connective tissue capsule was formed around each of the implants. The capsule was highly cellular, containing many young fibroblasts, histiocytes, and thin-walled capillaries. A syncytium of histiocytes was always found immediately adjacent to the surface of, and completely surrounded, each implant. A few delicate fibrils were seen between the fibroblasts of the capsule. The only differences in the connective tissue reactions were the presence of polymorphonuclear leukocytes in moderate numbers in the heterologous bone implant and in small numbers in the isologous bone and the anorganic bone implant capsules. These findings characterized the implants through the first week.

Little change occurred in the capsule during the first, second, and third months. There was some reduction in the cellularity of the capsule with maturation, characterized by increased collagenization and reduction in vascularity. Histiocytes, resembling foreign-body giant cells, still surrounded the surface of each implant. There was also a reduction of the polymorphonuclear leukocyte infiltration. Howship lacunae with giant cells were found on the isologous bone.

The capsule continued its maturation by increased collagenization and reduction in cellularity and vascularity during the fourth, fifth, and six months. At 6 months, the capsule was well collagenized. The polymorphonuclear leukocyte infiltration of the capsule was only slight in the heterologous bone and was absent in anorganic bone and isologous mouse bone. Also, the syncytium of histiocytes and foreign-body-type giant cells persisted on the surface of the implants. Rarely, Howship lacunae structures were observed on the isologous bone implant surfaces.

Reaction of the implant.—The matrix of anorganic bone, heterologous bone, and isologous bone contained mucpolysaccharide, as seen in sections stained by the P.A.S. method. In fact, this was seen in sections of all specimens examined throughout the investigation.

In sections stained by the von Kossa method for calcium, all the implants stained a

* Anorganic bone was made available through the courtesy of Armour Laboratories, Chicago, Illinois.

uniform black. At 3 days, anorganic bone appeared as dense black clumps, particles, and granules. The grandules represented the "dust" of the soft anorganic bone at the time of implantation (Fig. 1). The heterologous bone and isologous bone appeared as black spicules of lamellated bone tissue.

In sections of anorganic bone implanted for 1, 2, and 3 months, the dust particles were lost, and the anorganic bone appeared as orange-black particles. The periphery of the particles were transparent and blended into orange and black at the center, as seen in sections stained for calcium (Fig. 2). The isologous bone and heterologous bone stained uniformly black.

The sections of anorganic bone implanted for 4, 5, and 6 months stained by von Kossa showed considerable differences. The anorganic bone appeared as a transparent matrix with slight orange-black centers. In the case of smaller particles, the implant was completely transparent (Fig. 3). The heterologous bone implants stained a uniform black but showed at their periphery a narrow band of transparency. Isologous bone stained uniform black (Fig. 4).

DISCUSSION

The implantation of small particles of anorganic bone into the subcutaneous tissue of mice serves as a useful technique for studying the fate of the implant. Moreover, undecalcified sections of such implants serve to demonstrate the fate of the mineral elements.

The formation of osteoclasts did not occur in anorganic bone implants. Instead, a syncytium of histiocytes, which resemble osteoclasts, surrounded the anorganic bone implants. Possibly, the treatment with ethylene diamine alters the remaining organic matrix, which may resist resorption. In all cases of anorganic bone implants, the matrix remained, even after all the mineral was lost. Evidently, it is this residual matrix that serves to retain the bone organ form (Fig. 5).

The fibrovascular capsule, in addition to the histiocytes, effectively walled off implants of anorganic bone. The inorganic salts leached out of the implant into the fluid system of the capsule. No doubt, this was possible because the amount of organic matrix in anorganic bone could not effectively bind the salts in a tissue fluid medium. At no time, except during the initial implant, did salts diffuse beyond the limits of the capsule. The observation of the reduction in phosphate and the associated calcium in the implants as a function of time demonstrated mineral loss. In all the implants the loss of bone salts proceeded from the periphery. At the same time, no positive reaction for phosphate was seen in the histiocytes. The salts of implanted anorganic bone were lost by leaching out of the matrix and controlled vascular absorption within the capsule. The only changes in the capsule were an increase in collagenization associated with a reduction in cellularity (Figs. 6–8).

Heterologous bone implants followed essentially the same pattern as anorganic bone (Fig. 9). The only exceptions were an acute polymorphonuclear leukocytic infiltration associated with the heterologous bone implants. However, even with anorganic bone, slight polymorphonuclear leukocytic infiltration was seen. Resistance to resorption may be attributed to denaturation of protein due to boiling. A small amount of salts was leached out from the surface of heterologous bone, possibly as a result of hydrolyzing the cementing substance and the collagen.

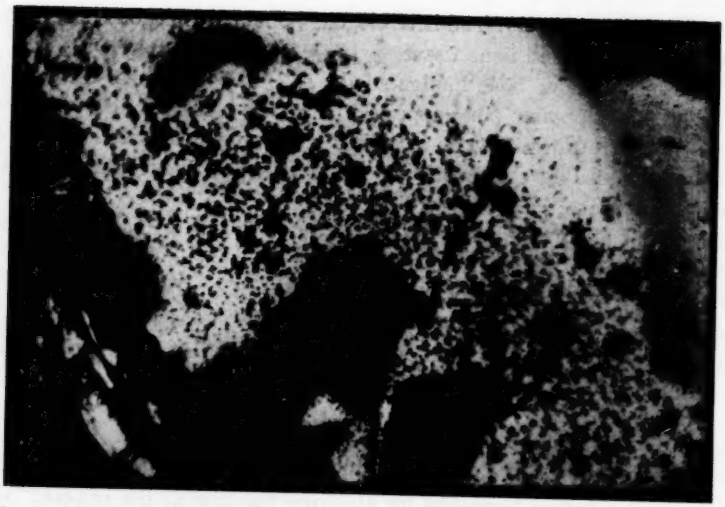

FIG. 1.—Anorganic bone implanted for 3 days, showing intense reaction. Note small "dust" particles, which were loosely held on the anorganic bone at the time of implantation. (von Kossa, mag. ×400.)

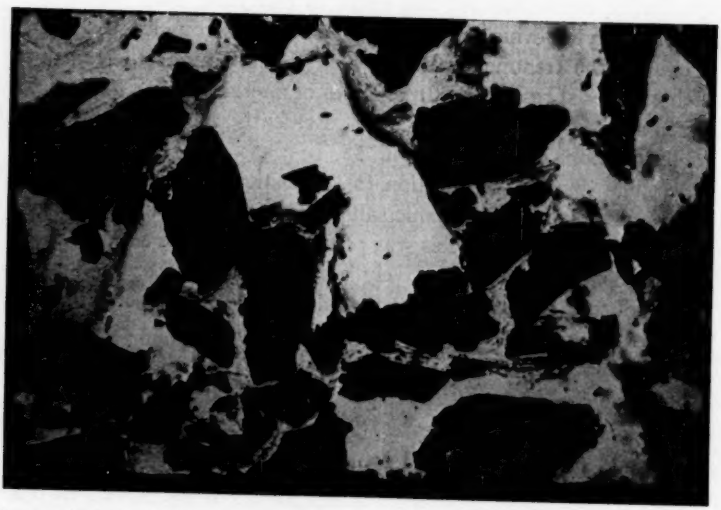

FIG. 2.—Anorganic bone implanted 1 month, showing intense reaction. Note the absence of the "dust" particles. (von Kossa, mag. ×400.)

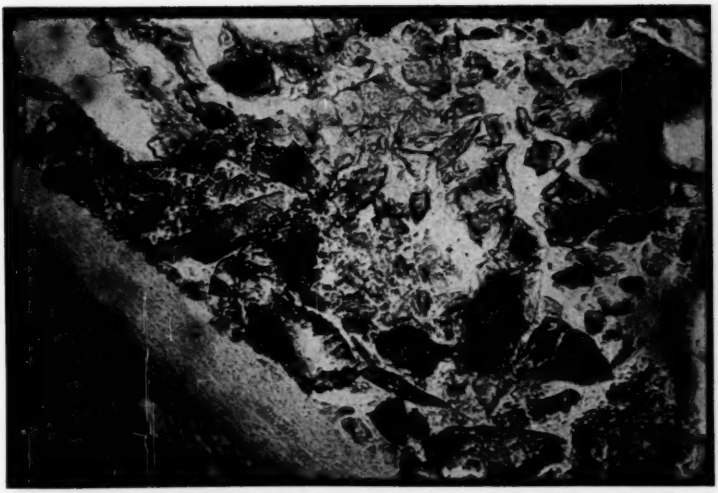

FIG. 3.—Anorganic bone particles implanted 6 months show loss of mineral. The matrix remains intact. Dense fibrosis of the capsule also is seen. (von Kossa, mag. ×100.)

FIG. 4.—Heterologous beef bone implanted 6 months shows slight loss of mineral from the periphery. The main mass is strongly positive. (von Kossa, mag. ×400.)

FIG. 5.—Anorganic bone implanted 6 months. Matrix contains mucopolysaccharides. Note fibrous capsule. (P.A.S., Coleman, mag. ×400.)

FIG. 6.—Anorganic bone implanted 3 months, showing capsule. (Particles of implant lost through preparation.) (H. & E., mag. ×25.)

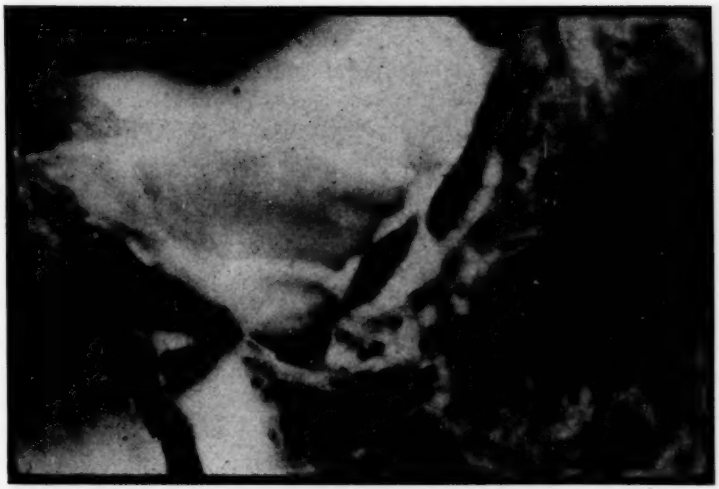

FIG. 7.—High power of Fig. 6, showing capsule and giant cells adjacent to anorganic bone implant. (H. & E., mag. ×400.)

FIG. 8.—Anorganic bone implanted 6 months, showing persistence of histiocytic syncytium and fibrosis of the capsule. (H. & E., mag. ×400.)

FIG. 9.—Heterologous beef bone implanted 6 months, showing giant cell and capsule. (H. & E., mag. ×400.)

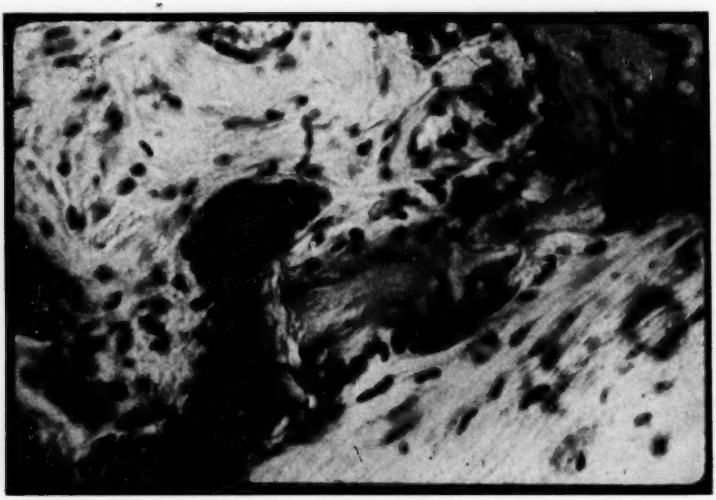

FIG. 10.—Isologous mouse bone implanted 6 months, showing giant cell and fibrous capsule. A giant cell in Howship's lacunae is shown at left center. (H. & E., mag. ×400.)

It seems clear that the implants caused a reaction comparable to a foreign-body reaction. The histiocytic syncytium and the capsule substantiate this interpretation. The polymorphonuclear leukocyte infiltration especially in heterologous bone also supports this interpretation. Isologous bone implants never demonstrated any loss of salts from the periphery. Howship lacunae containing giant cells suggested slow resorption of bone (Fig. 10). In this regard, it differed from heterologous bone and anorganic bone. Like anorganic bone, only slight inflammation was induced with isologous bone. Of the three implants, heterologous bone presented the most classic picture of the foreign-body reaction.

SUMMARY

Forty mice were used for the subcutaneous implantation of 40-mesh anorganic bone, heterologous boiled beef bone, and isologous mouse bone. Histologic study of undecalcified bone implant from 24 hours through 6 months was done. Special stains, von Kossa for calcium phosphates and P.A.S. for mucopolysaccharides, were used.

The anorganic bone was surrounded by a cellular and vascular capsule. Histiocytes were elaborated from undifferentiated connective tissue and came into immediate surface contact and enveloped the implant.

The mineral salts of the implant leached out into the tissue fluid, because of the reduced amount of anorganic matrix capable of binding the salts in a fluid system. In time, the periphery of the implant completely lost its mineral content, while the central areas still retained some. At 6 months, some implant particles were completely free of salts; yet the matrix remained surrounded by a syncytium of histiocytes and a capsule. Rarely was a surface defect in the implant resembling Howship lacunae seen. The capsule served to control the removal of leached-out salts and to wall off the matrix from the connective tissue of the implant site. In effect, the capsule was a physiologic barrier to regulate absorption of high concentration of minerals and a histologic barrier walling off foreign protein matrix.

Isologous mouse bone and heterologous beef bone induced a reaction similar to anorganic bone. The polymorphonuclear leukocytic infiltration which accompanied heterologous bone and persisted for 6 months appeared as a classic foreign-body reaction.

It was concluded that anorganic bone did not show any bone inducing properties. Moreover, it was not resorbed over a period of 6 months and therefore did not demonstrate any osteoclastic-inductive-property.

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An Impact Test for Dental Porcelain

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A search has been conducted over a period of years for a test that could be used to predict the clinical behavior of dental porcelains under impact forces. At least eight different tests used in other fields have been tried, such as the Charpy and Izod (swinging pendulum–flexure), and Olsen (dropping weight–flexure and compression), but all failed to yield results that were definitive in terms of dental serviceability. In early 1954, Mr. P. W. Lee, in further attempts at correlation, discovered that special geometric porcelain forms used in conjunction with a dropping-weight test showed distinctions between porcelains that could be related to their clinical behavior.

Concurrently with this development of a test technique, a vacuum-fired porcelain tooth had been developed, and clinical reports indicated it to be less brittle than either the translucent air-fired porcelain then on the market or the more opaque porcelains of former years that were also air-fired. Work was directed therefore toward refining and calibrating Mr. Lee's test to correlate with these findings. Work done by Vines, Semmelman, Lee, and Fonvielle[1] on porcelain densification predicted a stronger porcelain under impact forces, and this was another reason for development of such a test.

EXPERIMENTAL METHODS

In the technique proposed by Lee, impact forces are applied to porcelain specimens by means of a freely falling steel weight or "tup" of known hardness (Fig. 1), dropping within the confines of vertical guides marked in increments of 1 cm. to measure the height of fall (Fig. 2). The axis of the vertical guide chute is aligned by means of leveling foot screws on the base. The guide supports and specimen holder are constructed (Fig. 3) so as to allow the tup to deliver the impacting blow squarely upon the securely clamped specimen. Friction is minimized by chrome plating the inner area of the guide supports. The cylindrical tup has a hook on one end to facilitate lifting inside the chute (Fig. 4), and the impacting end is machined to a 16 micro-inch finish.

Several geometric specimen shapes were considered, but the two finally selected for study corresponded approximately to an incisal contour and a cuspal contour (Fig. 5). These conical forms were chosen as an approximation of incisal and cuspal configurations and because they could be duplicated repeatedly with mechanical accuracy. Since the brittleness of the specimen could be affected by temperature, all specimens were preconditioned and tested at 75° ± 5° F.

Preliminary investigations showed dental porcelain to be subject to minimum fatigue effects; so impacting was begun at heights well below the fracture point. One blow was

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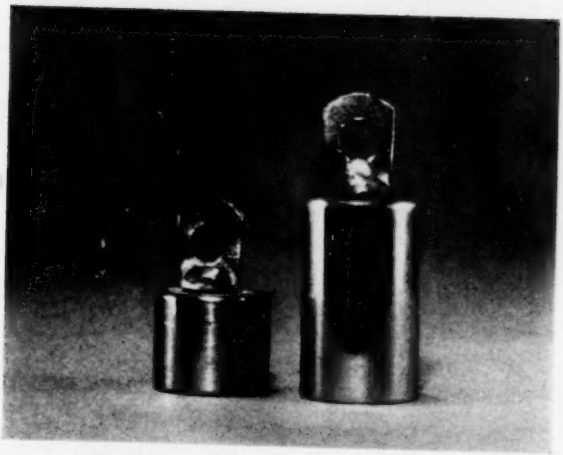

FIG. 1.—Steel impacting weights. *Left:* 17.5 gm.—Rockwell 12C; *right:* 25 gm.—Rockwell 42C

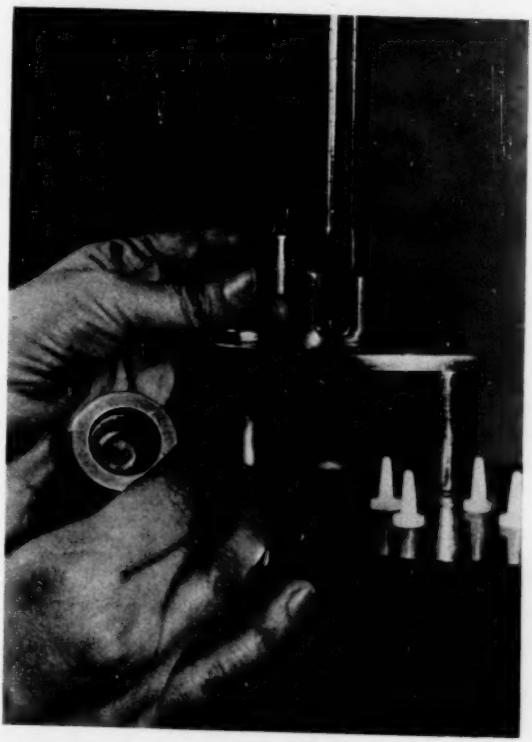

FIG. 2.—Impact test instrument, showing holder which allows a securely clamped specimen to receive a properly positioned blow.

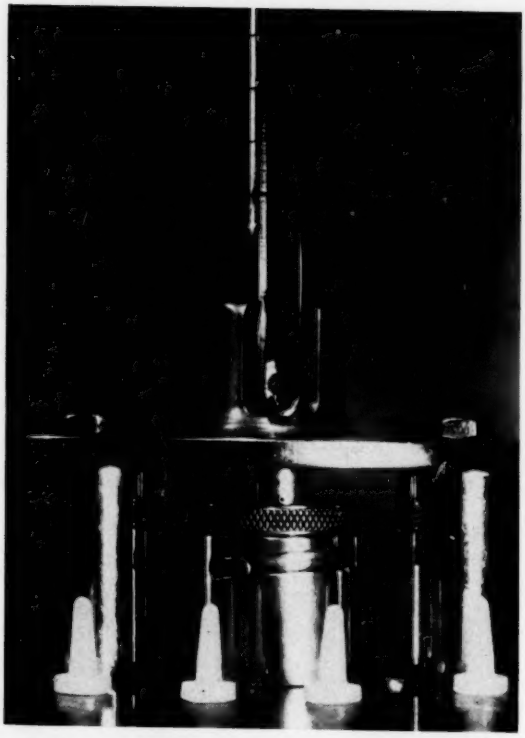

FIG. 3.—Impact test instrument showing weight at moment of impact upon specimen after a free fall from measured heights in the vertical chute.

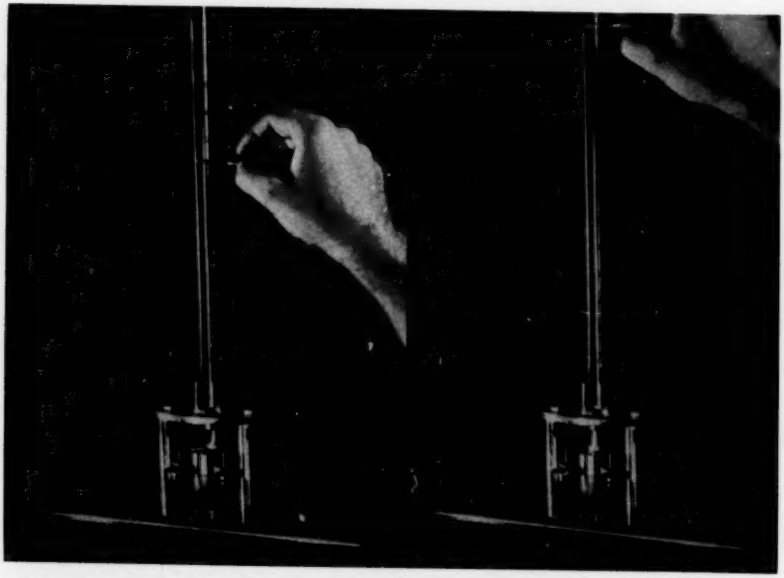

FIG. 4.—Impact test instrument showing method of elevating weights. A bluntly pointed tool engages a hook on the end of the weight for lifting to the desired heights.

delivered at each height, successive heights being increased each time by 1-cm. increments. Failure was determined by the initial sign of fracture regardless of its extent; however, the type or size of the fracture was also noted for purposes of data interpretation. Three categories of failure were observed (Fig. 6): slight damage or minute chipping of the tip, moderate destruction which included a splintering of the cone, and complete destruction where the cone was broken completely from the base.

Single-specimen data proved to be erratic because of the heterogeneous nature of dental porcelain. If the point of impact happened to be on a silica crystal, the resistance to impact was greater than if contact had been made upon the glassy matrix. Therefore, all data reported are, of necessity, the averages of a sample size of 25 specimens.

Imperfectly aligned specimens (Fig. 7) affected the test data. Straight cones yielded

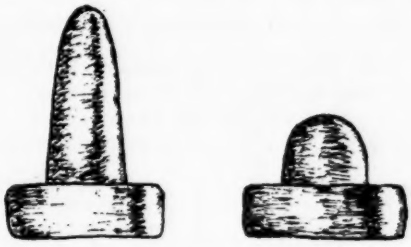

FIG. 5.—Geometrical porcelain shapes studied. *Left:* ¾ inch high, approximate incisal contour; *right:* ¼ inch high, approximate cuspal contour.

FIG. 6.—Types of failure under impact. *Left to right:* Slight damage, moderate destruction, complete destruction.

FIG. 7.—Degree of specimen angulation resulting from slumping during porcelain fusion. *Left to right:* Straight, angled, strongly angled.

significantly higher impact strengths than did angled specimens, as shown in Table 1. No statistical difference in test results was found between specimens having different amounts of angulation. But, since all angled specimens yielded lower averages and greater variability, as shown by the coefficient of variation, only straight specimens were used.

Since energy measurements are difficult to make accurately under impact conditions, such calculations were eliminated, as the height of fall was usually sufficient to differentiate between porcelains. When using different impacting weights, measurements were reduced to units of force expressed in gram-centimeters for direct comparisons.

Development of the test included extensive investigation into the effects of porcelain grain size, composition, firing variables, the shape of the impacting surface, surface finish of specimen bases, etc.

TABLE 1

EFFECT OF ANGLED SPECIMENS (¾ INCH) ON IMPACT STRENGTH, VACUUM-FIRED TRANSLUCENT ENAMEL

Degree of Angulation	Impact Strength Gram/Centimeters	
	Arithmetic Mean	Coefficient of Variation
Straight	420	27
Angled	290	60
Strongly angled	310	64

Various conditions of impact were studied, using tups of different weights and hardness to simulate a range of conditions that porcelain teeth could possibly encounter while in service. It was theorized that damaging impact could occur in service while a denture was being cleaned if it were dropped accidentally into a porcelain lavatory. To simulate this condition, a tup was used that was comparatively equal in hardness to porcelain enamel (Rockwell C42) and approximately equal in weight to an upper denture containing porcelain teeth (25 gm.). The slender cone-shaped ¾-inch specimens represented the vulnerable anterior incisals while the more blunt ¼-inch samples simulated the posterior cusps. In a subsequent test series a lighter-weight tup (17.5 gm.) of softer steel (Rockwell C12) was used, and preliminary checks indicated that such weight and hardness would produce more significant results. Routine comparisons have now been standardized to use a 17.5-gm. tup on incisal shapes, and such data can be reported simply in centimeters.

RESULTS

Table 2 shows a composite of average data obtained over many months of investigation. It includes various test conditions and different porcelains, the behavior of which were known from extensive service records. The average results show that impact strength as determined by this method was best defined by an incisal-shaped specimen when impacted by a 17.5-gm. weight. A confirming statistical Student's "*t*"-test

was performed on these data, and significant differences were found between all porcelains of the incisal–17.5-gm. weight series. This was not true of the other three series, as significant differences were found only in half the cases.

The greater density of vacuum-fired porcelain yielded a higher impact strength than air-fired porcelains. Increases due to this higher density varied from 50 to 117 per cent by the incisal–17.5 gm test method. There was a corresponding difference in the severity of the type of fracture, as air-fired specimens were usually shattered, while the average vacuum-fired specimen suffered only minor chipping.

A recent field report on laboratory-made jacket crowns corroborates the above data, for, on some 8,000 jacket crowns made by both the air- and vacuum-firing techniques, the remakes necessary due to service breakage were: air, 5 per cent; vacuum, 1 per cent.

TABLE 2

IMPACT RESISTANCE IN GRAM/CENTIMETERS, PORCELAIN

Condition of Test	Air-fired Translucent Enamel	Vacuum-fired Translucent Enamel	Vacuum-fired Translucent Dentin	Air-fired Opaque
Incisal shape; 17.5 gm. tup—Rockwell 12C.....	225	420	490	280
Incisal shape; 25 gm. tup—Rockwell 42C.....	135	210	245	175
Cuspal shape; 17.5 gm. tup—Rockwell 12C.....	281	438	473	434
Cuspal shape; 25 gm. tup—Rockwell 42C.....	351	402	450	376

DISCUSSION

Davis, Troxell, and Wiskocil[2] state that each type of impact test has its own particular area of use and thus its value lies in its connection with field-service performance. Data obtained by the present test method (incisal shape–17.5-gm. weight) show that air-fired translucent porcelains are more brittle than air-fired opaque porcelains and that vacuum-fired porcelains are less brittle than either. This agrees with field-service records. Air-fired translucent porcelain was more brittle than the earlier opaque porcelain which was also air-fired. The translucent air-fired porcelains apparently were accepted because of their better appearance in spite of their brittleness. Field breakage of present vacuum-fired porcelain teeth due to impact is so reduced as to be virtually nonexistent.

SUMMARY

A dropping-weight impact test for dental porcelain has been developed which correlates qualitatively with observed clinical behavior. Significant impact-strength differences were found between porcelains, the extent or degree of the differences being controlled by the specimen shape and exact test conditions. Vacuum-fired porcelains were consistently stronger than air-fired porcelains when measured by this test method.

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The Effects of Continued Exposure to Added Increments of Water-borne or Food-borne Fluoride upon the Rat

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Information continues to be accumulated from investigations of human populations which have daily imbibed waters containing various low concentrations of fluoride (F^-) for extended periods of time with no apparent adverse effects.[1, 2] In contrast, substantial data have been reported in recent years for livestock on the obviously adverse effects of long-term ingestion of chronically toxic dosages of F^-.[3] However, few experiments have been designed to evaluate the effect of time in F^- toxicity with laboratory animals,[4, 5] although a number of studies have been reported in which F^- has been provided for considerable periods of time.[6–10]

There have been reports that equilibrium between intake and output of F^- is quickly attained in human subjects during continuous ingestion of small amounts of F^-[11] and that in rats the percentage of ingested F^- which is excreted increases steadily during continuous exposure.[12] Others have reported, however, that F^- concentrations in human bone continue to increase with age for many years under these conditions.[13, 14] Similarly, in this laboratory, studies have demonstrated without exception that livestock (cattle, swine, and poultry) and laboratory animals (rats, guinea pigs, and rabbits) rapidly store F^- in their skeleton during growth and continue to accumulate additional F^- for extended periods of time. These studies covered a wide range of F^- administration. Bone deposition was found to be mainly dependent on these factors: the type and character of the bone, the level of F^- ingestion, the duration and continuity of exposure, and the age (in relation to bone growth) at which F^- ingestion was begun.

The purpose of these studies was to obtain additional data on the effects of various dosages of soluble F^- (NaF) in food and water on skeletal and dental accumulation of F^- under conditions of long-term exposure.

EXPERIMENTAL METHODS

Six groups of 25 Sprague-Dawley male and female weanling albino rats weighing between 45 and 55 gm. were fed a seminatural, moderately cariogenic diet ad libitum

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continuously up to 21 months. Four animals, 2 males and 2 females, were removed from each group at 3, 6, 12, and 18 months and the balance at 21 months. All groups received distilled water and the basal cariogenic diet to which increments of NaF were added according to the following plan: Lot 1—control, basal diet and distilled water (DW), no F^- added; Lot 2—1.2 p.p.m. F^- in DW; Lot 3—3.0 p.p.m. F^- in the basal diet; Lot 4—20.0 p.p.m. F^- in DW; Lot 5—45.0 p.p.m. F^- in the basal diet; and Lot 6—90.0 p.p.m. F^- in the basal diet. Seventeen additional weanling rats were divided into 3 groups of 5 each and given the same F^- supplements as Lots 3, 4, and 5. One rat from each group was removed after 10, 20, 30, 50, and 70 days of F^- treatment. The 2 remaining rats were sacrificed before F^- treatment to determine initial F^- content of the femurs. The femurs and heads were removed from all the animals for dental scoring and F^- analysis.

The percentage composition of the basal diet was as follows: oat groats, finely ground, 25; sucrose, 23.65; soybean oil meal, solvent-extracted, 22; dry skim-milk powder, 20; corn oil, 5; dried brewer's yeast,* 3; $CaHPO_4 \cdot 2H_2O$, A.R., 0.85, and NaCl, iodized A.R., 0.50. Analysis of the basal diet indicated a F^- content of approximately 0.5 p.p.m. (dry-weight basis). Halibut liver oil was administered weekly as a source of the fat-soluble vitamins.

The criteria used to evaluate the long-term effects of added increments of NaF were general health and well-being, growth rate, maintenance of body weight, reproductive performance, femur F^-, molar and incisor F^-, and its effects on dental caries, periodontal disease, molar wear, and enamel structure.

Male and female rats were housed in separate groups in coarse screen-bottomed metal cages. Weekly weighings were made, and individual records were kept on body weight and general health. Food- and water-consumption data were taken at three different periods during the experiment: at the beginning, at 1 month, and at 3 months (Table 1). Data on dietary consumption during pregnancy and lactation had been obtained from previous experiments.[5] Arbitrarily, reproduction was begun at approximately 4 months and terminated at a little over 12 months. The female rats were allowed to reproduce as frequently as possible and were given no rest period between weaning of the young and rebreeding. Records were kept of the apparent number of pregnancies, the number of litters, the number of live and dead young per litter, the weight of the newborn and weanling litters, and the percentage of the litters weaned.

The enamel and dentin were quantitatively separated by Gilda's modification[15] of the flotation technique of Manly and Hodge.[16] The enamel, dentin, and bones were analyzed for F^- by a modification of the Willard and Winter method.[17] Dental caries scores were determined by a modification of the method of Shaw, Schweigert, McIntire, Elvehjem, and Phillips.[18] Periodontal lesions were scored by determining the incidence of lesions per rat and assigning a numerical value from 1 to 5 to the severity of each lesion. The wear on the molars and the severity of dental fluorosis of the incisor enamel were evaluated both by visual scoring and by determining the percentage of enamel obtained by separation.

* We are indebted to the Joseph Schlitz Brewing Co., Milwaukee, Wisconsin, for supplying the dried brewer's yeast, and to Merck, Sharpe, and Dohme, Research Laboratories, Rahway, New Jersey, for the vitamins used.

RESULTS

No marked effects of F^- were observed on growth rate or on general health. Slight temporary retardations of growth rate were observed in the rats receiving 90.0 p.p.m. F^- in food. Generally inferior reproductive performance was observed in the rats receiving the three higher F^- levels. In these high F^- rats there were 7 maternal deaths at parturition out of 25 rats. This compared with only 2 out of 25 in the low F^- females. Taking these deaths into consideration, there was still a reduction in the average number of surviving litters per female and in the total number of surviving young per female. There were no differences between low and high F^- groups in the average number of pups per litter, in the average birth weight or weaning weight of the litters, or in the percentage of the young weaned. No gross defects were observed in the skeletal structures of the experimental animals at the levels of F^- used in this study.

Progressive increases in the F^- content of the bones and teeth occurred with age as a result of administering both food- and water-borne F^- supplements (Tables 2–4). Dry-weight F^- concentrations increased most rapidly at the beginning of treatment but continued to rise throughout the experimental period (Fig. 1). Regardless of whether water or food was used to administer the F^-, during growth the amount of F^- deposited in the femurs and teeth was directly proportional to the rate of F^- intake (i.e., the μg of F^- consumed per day/gram of body weight). Concentration-

TABLE 1

FOOD AND WATER CONSUMPTION

F^- Additions	Sex	Day 1–10				Day 28–36				Day 97–101			
		No. Rats	Food* (Gm.)	Water* (Gm.)	W/F†	No. Rats	Food (Gm.)	Water (Gm.)	W/F	No. Rats	Food (Gm.)	Water (Gm.)	W/F
None	F	13	7.0	12.0	1.7	13	11.0	25.1	2.3	11	10.2	26.4	2.6
	M	12	7.4	13.3	1.8	12	15.0	29.3	2.0	10	17.6	37.6	2.1
1.2 p.p.m. in dist. water	F	12	7.1	11.5	1.6	12	11.3	23.2	2.0	10	10.5	28.0	2.7
	M	13	7.3	11.5	1.6	13	14.2	26.8	1.9	11	17.4	34.2	2.0
3.0 p.p.m. in food	F	13	7.3	12.3	1.7	13	12.7	26.7	2.1	11	11.4	27.9	2.5
	M	12	8.7	12.6	1.5	12	16.4	26.4	1.6	10	18.4	35.3	1.9
20.0 p.p.m. in dist. water	F	13	6.9	12.0	1.8	12	10.9	24.9	2.3	11	11.2	28.3	2.5
	M	12	7.6	12.7	1.7	12	16.5	29.0	1.8	10	17.5	32.9	1.9
45.0 p.p.m. in food	F	12	6.9	12.0	1.8	12	10.9	24.9	2.3	10	10.2	27.3	2.7
	M	13	7.8	12.2	1.6	13	15.3	28.0	1.8	11	18.5	34.1	1.8
90.0 p.p.m. in food	F	12	6.7	13.0	1.9	12	11.2	22.8	2.0	10	10.5	25.9	2.5
	M	13	7.6	12.1	1.6	13	15.3	26.9	1.8	11	18.9	32.3	1.7
Av. of all groups	F	75	7.0	12.1	1.7	74	11.7	24.2	2.1	63	10.6	27.3	2.6
	M	75	7.7	12.4	1.6	75	15.4	27.7	1.8	63	18.0	34.3	1.9

* Food and water consumption in grams per day.

† Water consumption/food consumption.

TABLE 2

EFFECT OF TIME ON F^- ACCUMULATION IN FEMURS OF RATS GIVEN VARIOUS F^- LEVELS IN FOOD AND WATER

F^- Additions	Sex	F^- Concentration in Femur (Dry Weight)														
		94 Days			184 Days			365 Days			554 Days			622 Days		
		No. Rats	Femur Wt. (Mg.)	F^- Conc.* (p.p.m.)	No. Rats	Femur Wt. (Mg.)	F^- Conc. (p.p.m.)	No. Rats	Femur Wt. (Mg.)	F^- Conc. (p.p.m.)	No. Rats	Femur Wt. (Mg.)	F^- Conc. (p.p.m.)	No. Rats	Femur Wt. (Mg.)	F^- Conc. (p.p.m.)
None	F	2	459	23	2	520	47	3	457	84	2	524	66	2	610	60
	M	2	634	22	2	781	34	3	785	57	2	836	40	0	.....	.....
1.2 p.p.m. in dist. water	F	2	464	114	2	518	160	2	470	318	2	565	306	2	614	278
	M	3	599	108	2	784	146	3	805	175	2	778	178	2	902	198
3.0 p.p.m. in food	F	2	460	134	2	537	187	1	498	400	3	563	329	3	560	332
	M	3	640	145	2	780	148	3	753	237	1	786	230	2	872	270
20.0 p.p.m. in dist. water	F	2	484	1395	3	452	2575	2	584	2950	1	591	3170	2	616	3240
	M	3	632	1405	3	745	1900	3	806	2270	3	810	2225	0	.....	.....
45.0 p p.m. in food	F	2	448	1735	4	505	2250	3	546	3525	1	670	2880	2	630	3330
	M	2	618	1670	2	746	2205	2	861	2575	4	803	2970	3	857	2655
90.0 p.p.m. in food	F	2	410	3230	3	504	4260	2	521	5825	2	557	5820	2	560	6095
	M	2	652	3465	2	787	3660	6	791	4645	3	813	5050	0	.....	.....

* Standard errors of the F^- concentrations averaged 5.1 ± 0.5 per cent of the tabulated means.

TABLE 3—EFFECT OF TIME ON F⁻ ACCUMULATION IN MOLAR TEETH OF RATS GIVEN VARIOUS F⁻ LEVELS IN FOOD AND WATER

F⁻ Additions	Tissue	Sex	F⁻ Concentration in Molar Teeth (Dry Weight)														
			94 Days			184 Days			365 Days			554 Days			622 Days		
			No. Rats	Tissue Wt.* (Mg.)	F⁻ Conc.† (p.p.m.)	No. Rats	Tissue Wt. (Mg.)	F⁻ Conc. (p.p.m.)	No. Rats	Tissue Wt. (Mg.)	F⁻ Conc. (p.p.m.)	No. Rats	Tissue Wt. (Mg.)	F⁻ Conc. (p.p.m.)	No. Rats	Tissue Wt. (Mg.)	F⁻ Conc. (p.p.m.)
None	En.	F	2	19	34‡	2	21	21	3	17	30	2	12	28	2	15	34
		M	2	22	34	2	23	21	3	19	30	2	14	28	0	.....	.....
	Den.	F	2	87	33	2	98	23	3	101	37	2	91	42	2	104	40
		M	2	94	17	2	106	34	3	117	23	2	93	28	0	.....	.....
1.2 p.p.m. in dist. water	En.	F	2	17	31	2	22	25	2	16	58	2	21	51	2	16	44
		M	3	21	30	2	18	36	3	19	32	2	17	31	2	16	58
	Den	F	2	88	69	2	101	97	2	101	214	2	107	288	2	105	382
		M	3	93	80	2	95	92	3	106	133	2	130	155	2	117	221
3.0 p.p.m. in food	En.	F	2	19	40	2	28	35	1	16	47	3	14	46	3	14	48
		M	3	27	44	2	24	38	3	17	42	1	18	39	2	18	37
	Den.	F	2	94	144	2	100	172	1	91	251	3	113	334	3	115	300
		M	3	93	129	2	104	168	3	106	231	1	106	263	2	121	228
20.0 p.p.m. in dist. water	En.	F	2	18	222	3	22	235	2	20	276	1	20	310	2	18	252
		M	3	22	206	3	21	298	3	20	230	3	21	248	0	.....	.....
	Den.	F	2	76	1020	3	101	1630	2	111	2210	1	132	3060	2	106	2965
		M	3	89	1030	3	99	1265	3	113	1690	3	118	2005	0	.....	.....
45.0 p.p.m. in food	En.	F	2	21	236	4	18	307	3	19	320	1	16	407	2	13	390
		M	2	21	298	2	21	330	2	20	355	4	15	299	3	12	412
	Den.	F	2	93	1060	4	105	1775	3	110	2710	1	119	2860	2	107	2780
		M	2	98	1160	2	110	1510	2	112	2110	4	121	2480	3	110	2235
90.0 p.p.m. in food	En.	F	2	24	310	3	22	434	2	21	505	2	14	640	2	10	580
		M	2	21	378	2	20	630	6	19	387	3	8	481	0	.....	.....
	Den.	F	2	102	2100	3	104	2805	2	113	4390	2	104	4420	2	95	4880
		M	2	105	2160	2	104	2630	6	112	3540	3	99	4150	0	.....	.....

* Tissue weight is the mean value of the animals in each age and sex group.

† F⁻ concentration is from the analysis of pooled tissues from rats in each age and sex group.

‡ Enamel samples from male and female rats of the control group were pooled in each age group to obtain sufficient material for F⁻ analysis.

TABLE 4—EFFECT OF TIME ON F⁻ ACCUMULATION IN INCISOR TEETH OF RATS GIVEN VARIOUS F⁻ LEVELS IN FOOD AND WATER

ACCUMULATION IN INCISOR TEETH OF RATS GIVEN VARIOUS F⁻ LEVELS IN FOOD AND WATER

F⁻ Additions	Tissue	Sex	F⁻ Concentration in Incisor Teeth (Dry Weight)														
			94 Days			184 Days			365 Days			554 Days			622 Days		
			No. Rats	Tissue Wt.* (Mg.)	F⁻ Conc.† (p.p.m.)	No. Rats	Tissue Wt. (Mg.)	F⁻ Conc. (p.p.m.)	No. Rats	Tissue Wt. (Mg.)	F⁻ Conc. (p.p.m.)	No. Rats	Tissue Wt. (Mg.)	F⁻ Conc. (p.p.m.)	No. Rats	Tissue Wt. (Mg.)	F⁻ Conc. (p.p.m.)
None	En.	F	2	26	10‡	2	33	5	3	42	6	2	56	5	2	50	4
		M	2	30	10	2	34	5	3	47	6	2	52	5	0	.....	.....
	Den.	F	2	178	6	2	237	10	3	288	11	2	353	14	2	317	14
		M	2	236	6	2	259	6	3	319	6	2	371	7	0	.....	.....
1.2 p.p.m. in dist. water	En.	F	2	26	22	2	39	32	2	42	32	2	47	24	2	47	16
		M	3	32	22	2	39	24	3	38	23	2	45	21	2	59	18
	Den.	F	2	188	61	2	238	85	2	301	94	2	373	79	2	342	64
		M	3	254	75	2	309	80	3	280	60	2	391	53	2	494	66
3.0 p.p.m. in food	En.	F	2	29	16	2	38	16	1	49	25	3	52	23	3	46	24
		M	3	34	17	2	44	18	3	52	18	1	53	18	2	60	19
	Den.	F	2	192	73	2	244	99	1	293	132	3	389	90	3	322	90
		M	3	256	61	2	272	105	3	376	76	0	.....	.....	2	427	82
20.0 p.p.m. in dist. water	En.	F	2	25	246	3	27	414	2	38	321	1	42	256	2	30	345
		M	3	30	221	3	39	259	3	45	173	3	43	261	0	.....	.....
	Den.	F	2	164	851	3	254	1520	2	298	1290	1	369	1055	2	349	1450
		M	3	232	684	3	242	901	3	360	771	3	367	925	0	.....	.....
45.0 p.p.m. in food	En.	F	2	27	216	4	27	398	3	29	476	1	34	259	2	34	294
		M	2	28	240	2	41	214	2	41	261	4	36	258	3	33	212
	Den.	F	2	209	856	4	269	1465	3	300	1775	1	350	1190	2	305	1180
		M	2	240	857	2	306	907	2	406	896	4	387	1070	3	428	912
90.0 p.p.m. in food	En.	F	2	26	434	3	25	674	2	28	963	2	23	880	2	19	992
		M	2	21	534	2	30	583	6	26	614	2	20	595	0	.....	.....
	Den.	F	2	231	1840	3	278	2380	2	387	2580	2	362	2735	2	350	2570
		M	2	291	1830	2	350	1940	6	377	1605	2	449	1630	0	.....	.....

* Tissue weight is the mean value of the animals in each age and sex group.

† F⁻ concentration is from the analysis of pooled tissues from rats in each age and sex group.

‡ Enamel samples from male and female rats of the control group were pooled in each age group to obtain sufficient material for F⁻ analysis.

wise, however, a p.p.m. of F^- given in water would provide about twice as much F^- as a p.p.m. given in food because of the differences in food and water intake (Table 1). As age increased, the concentration of F^- attained in the femurs and teeth of the rats receiving the higher levels of F^- were somewhat less than proportional to the concentrations attained from the lower levels of F^- intake.

Rather obvious differences between sexes occurred in F^- storage. A distinct elevation in the F^- concentration of the bones and teeth was noted in the female rats which reached a peak of about 40 per cent or more several months after reproduction began (Fig. 1). No increase in femur F^- concentration was observed in the 12 females sacrificed before reproduction began. In 12 females sacrificed shortly after having been pregnant but not suckling their young to weaning age, an average increase of 21 per cent over the males of comparable age was observed. However, in 16 females which were sacrificed shortly after weaning an average of 21 young each, an average increase of 43 per cent was observed. These differences were apparently produced by the increased consumption of food and water by the females during pregnancy and especially during lactation. In the males, two distinct phases in the rate of femur F^- deposition were observed. The first was associated with bone growth; the second was

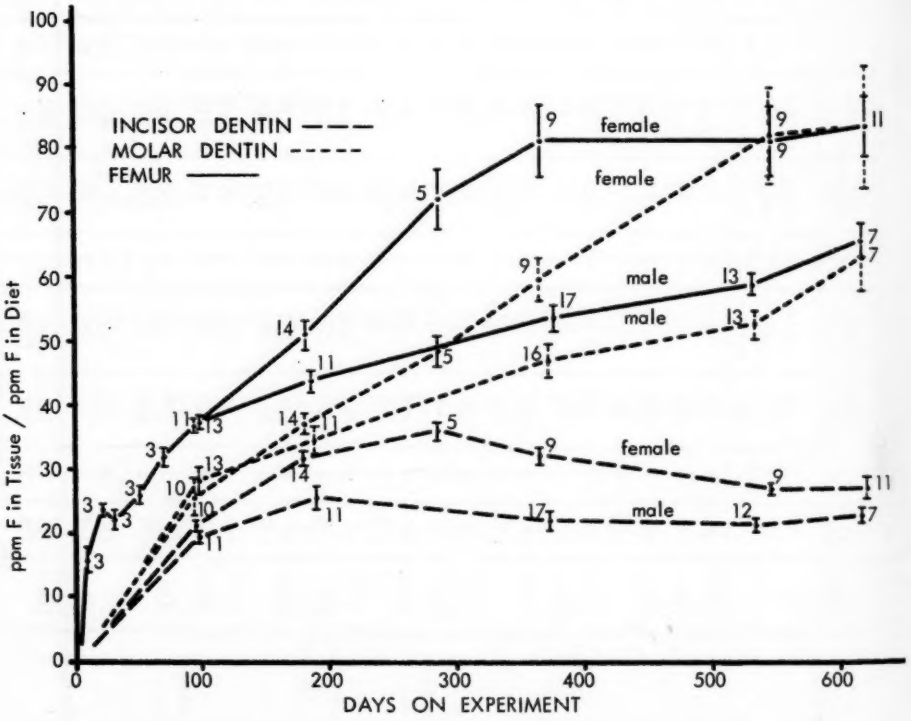

FIG. 1.—Sex difference in F^- storage in the femur, molar dentin, and incisor dentin. (Composite of data from all F^--treated animals.) F^- concentrations in the tissues are amounts in excess of those from control rats of similar age and are divided by the concentration of F^- in the food and twice the F^- concentration in water to equalize the differences in F^- intake. The number beside each point indicates the number of animals involved. Cross-bars indicate the standard errors of the mean values.

a gradual, more linear increase in concentration with time. The increased deposition observed in the females during reproduction was superimposed upon this second phase of F^- storage.

In the molar dentin (which included both primary and secondary dentin), F^- deposition was initially lower than in the femur, but there was a steadier increase in concentration and less difference between sexes (Fig. 1). Molar enamel displayed only a small initial increase in F^- concentration, and no sex differences were observed. The final enamel concentrations were roughly one-eighth of those of the dentin. The marked increase with age in molar dentin F^- concentration was probably caused in part by an increased proportion of secondary dentin caused by attrition of the primary dentin. In the incisors, F^- concentrations of the male rats were relatively constant after the first measurement at 94 days. However, the concentrations of the incisors of the female rats were clearly elevated during reproduction (Fig. 1). These incisor F^- levels probably indicated the rate of F^- intake during the previous few weeks quite accurately. The enamel F^- concentration in the incisors was only about one-fourth to one-third the concentration of the dentin.

Concerning the various levels of F^- administered to the rats in this experiment, the basal diet, which contained only 0.5 p.p.m. or less of F^- (dry weight), caused measurable small increments of F^- to be deposited in the femurs and teeth. However, the maximum concentrations were obtained at 1 year. Intake of F^- concentrations recommended by the U.S.P.H. for fluoridation of public water supplies for humans caused a five- to sixfold increase in the concentration of F^- in the calcified tissues, but it is important to note that the levels of F^- eventually attained were far below the toxic range. Intake of 90 p.p.m. of F^- in food by female rats for a period of 1 year resulted in F^- levels in the femurs which produce changes in compact bone and systemic F^- reactions in dairy cattle.[19]

The dental effects observed in this relatively caries-resistant strain of rats were as follows. Very few lesions were observed in any of the rats sacrificed prior to 1 year of F^- treatment. Dental caries was still not extensive; however, carious teeth were observed in 33 per cent of the rats receiving 20 or more p.p.m. of F^-. In the same age group, only 8 per cent of the rats receiving the low levels of F^- had dental caries. The percentage of teeth affected likewise increased much more rapidly in the high F^- rats than in the low F^- rats (Fig. 2). While these observations were not in line with reports from short-term studies in which reductions in the caries incidence were consistently obtained by use of low F^- levels, a similar occurrence has been reported by Ramseyer, Smith, and McCay.[4] However, in our investigation it is important to understand that the lesions were observed in the secondary dentin exposed by the attrition of the occlusal surfaces of the molars and were not seen in the enamel (Fig. 3). Hence our findings should not be assumed to have direct application to caries customarily found in humans.

Added increments of F^- supplementation caused progressive hypoplasia of the incisor enamel. Cross-striations similar to those described by Schour and Smith[20] and by Dean, Sebrell, Breaux, and Elvove[21] were observed. Quantitative separation of the incisor enamel and dentin revealed a measurable hypoplasia of the incisor enamel. No decrease in incisor dentin formation was observed. Further, the percentage of enamel in the incisors of the control rats gradually increased with time, whereas a propor-

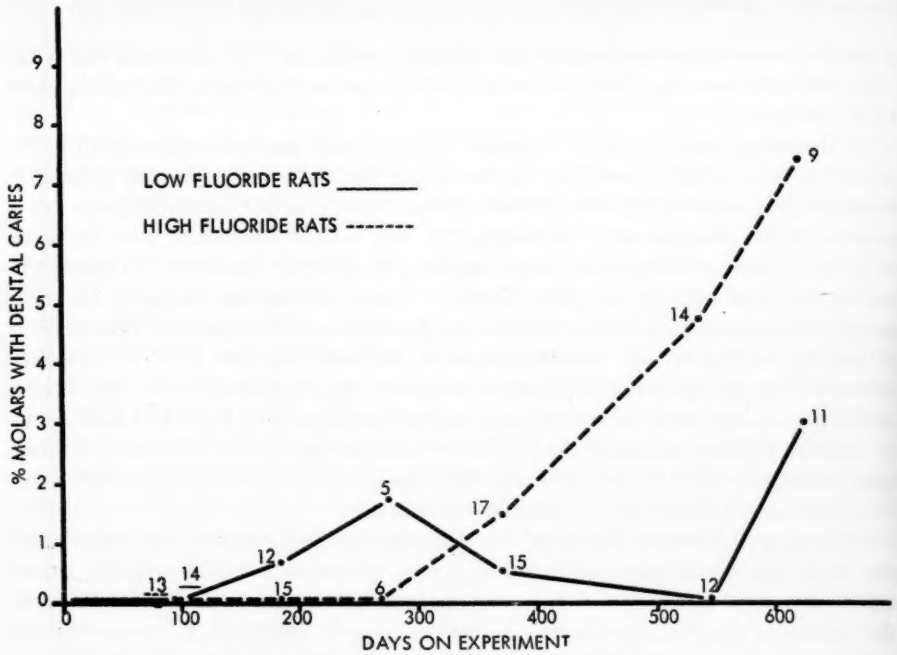

FIG. 2.—Effect of moderate intakes of F^- on the increase in dental caries of rats with age. Low-fluoride rats include the controls, those given 1.2 p.p.m. F^- in water, and those given 3.0 p.p.m. F^- in food. High-fluoride rats include those given 20.0 p.p.m. F^- in water and 45.0 and 90.0 p.p.m. F^- in food. The number beside each point indicates the number of animals involved.

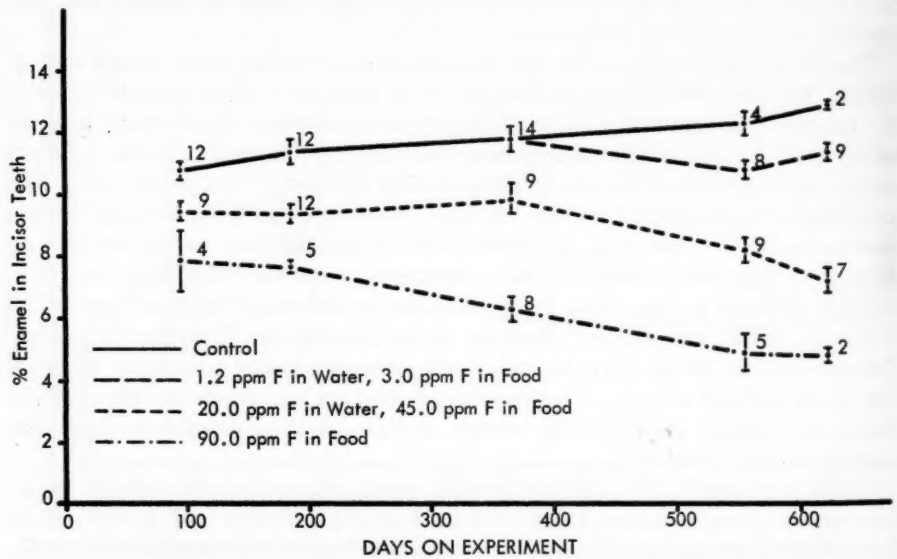
% Enamel in Incisor Teeth
Control
1.2 ppm F in Water, 3.0 ppm F in Food
20.0 ppm F in Water, 45.0 ppm F in Food
90.0 ppm F in Food
DAYS ON EXPERIMENT

FIG. 3.—Effect of increased F^- intake and age on enamel hypoplasia in incisor teeth of rats. The number beside each point indicates the number of animals involved. Cross-bars indicate the standard errors of the mean values.

tionate decline occurred in the F^--treated rats. (Fig. 4). At the termination of the experiment at 622 days, the percentage of enamel in the incisors of the rats receiving the low levels of added F^- were 88 per cent of the control values, those of the rats fed the intermediate levels were approximately 56 per cent, and those of the rats receiving 90 p.p.m. F^- in food were only 38 per cent of the control values. No influence was observed on the progress of alveolar bone resorption or on the rate of molar wear from these levels of F^- addition.

FIG. 4.—Site of "caries" lesion in the secondary dentin of the occlusal surface of the second cusp of the left first maxillary molar of female rat No. 11 exposed to 45.0 p.p.m. F^- in food for 622 days. Note that the entire tooth and some alveolar bone are now affected.

DISCUSSION

Shaw, Schweigert, Phillips, and Elvehjem demonstrated that dental caries in the cotton rat is reduced in a manner similar to that in the albino rat by graded levels of dietary F^-.[22] They found that 45, 90, and 135 p.p.m. F^- in food were needed to produce reductions in the incidence of dental caries of approximately 25, 65, and 90 per cent, respectively, in short posteruptive assay periods. However, McClure has reported that lower levels of F^- were effective when treatments were begun pre-eruptively.[23] The effects on the rat produced by exposure to graded levels of F^-, ranging from the fluoridation levels to the posteruptive levels for the rat just described, were interesting in several respects. All added increments of F^- resulted in progressive accumulation of F^- in the femurs and molar dentin. F^- uptake was most rapid during early ex-

posure to F^-, but gradual increases in the dry-weight concentration of F^- continued throughout the 622-day experimental period. Nonetheless, F^- ingestion at levels recommended for fluoridation of potable water supplies did not produce excessive storage levels in the bones and teeth of the rat. When F^- concentrations of 20 p.p.m. in water or 45 p.p.m. in food were used, hypoplastic effects on the incisor enamel were produced, and an increase in occlusal caries was observed after extended treatment. Increasing the dietary level of F^- to 90 p.p.m. in food produced a further intensification of enamel hypoplasia, produced a similar caries increase, and raised femur F^- concentrations into a range which was toxic in other species, although no gross defects were produced in these rats.

Thus the total daily intake of F^- from all sources appears to be of primary importance in assessing the likelihood of a fluorosis syndrome. The length of exposure time, the continuity of administration, the total F^- intake, the age and state of growth, the sex, species, and metabolic state of the skeletal structure under test modify the effects of F^-, but toxicity seems to depend to the greatest extent on the daily intake of F^-.

In attempting to understand the unexpected result of increased dental caries following prolonged exposure to moderate levels of F^- and to question whether these lesions were indeed true caries, it is important that the route of invasion was through the secondary dentin of the occlusal surface of the molars and not the enamel. This secondary dentin would be expected to be higher in F^- than primary dentin. The levels of F^- administration from which these increases in dental caries occurred may have produced excessive concentrations of F^- and thereby made the secondary dentin more susceptible to carious invasion, softer and more easily penetrated, or may have reduced the rate of secondary dentin formation. Regardless of the nature of the lesion, since human dentition normally has no exposed dentin surfaces, this problem has no human counterpart. However, one should note the teeth in the low F^- and control rats were sound, despite their low F^- content. This fact points out that there are obviously important factors other than F^- involved in the protection of teeth of rats from dental caries.

SUMMARY

The effects of administering various low dosages of water- and food-borne F^- to rats for extended periods of time have been investigated. These studies showed the following results. All levels of added F^- resulted in an increase in F^- content of the femurs and teeth. The amount of F^- deposited was directly proportional to the daily intake of F^- (μg F^-/gm body weight), regardless of whether it was given in food or water. However, from the viewpoint of dietary level, a concentration of F^- in water caused approximately the same F^- deposition as twice this level in food because water intake was double that of food. The F^- concentration in the femur and molar dentin gradually increased during the long treatment period. Of the tissues analyzed, F^- deposition was highest in the femur. Lesser amounts were found in the molar dentin, incisor dentin, and the enamel, in that order. A sex difference in F^- retention was described which appeared to be associated with the increased nutritional requirements imposed by pregnancy and lactation.

Contrary to the results of short-term experiments, extended exposure of the rat to

moderate dosages of F^- was accomplished by a significant increase in what we have considered to be dental caries. These lesions, however, were present in the secondary dentin of the occlusal surfaces of the molars and do not appear to be analogous to human caries. Sound teeth were developed in the absence of supplementary F^- on a ration containing 0.5 p.p.m. F^-. However, it should be noted that these animals were from a relatively caries-resistant strain and fed a moderately cariogenic diet.

Added increments of F^- caused progressive increases in incisor enamel hypoplasia. Only mild hypoplasia occurred in the rats receiving F^- supplements of 1.2 p.p.m. in water and 3.0 p.p.m. in food. These low intakes of F^- produced F^- levels in the calcified tissues which were well below the toxic range for this species.

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Relative Frequency of Loss of Individual Molars of Rats during a Longevity Study

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Numerous workers have reported on the variation in incidence of caries with position of the molars in the jaws of rats. Hunt and Hoppert[1] observed 3,779 carious lesions in the lower molars of their resistant strain and 6,269 carious lesions in the corresponding teeth of their susceptible strain of rats and reported the distribution of caries in the first, second, and third molars. Shaw, Schweigert, McIntire, Elvehjem, and Phillips[2] observed both the upper and lower molars of 65 cotton rats and reported the incidence of caries for each molar. Sognnaes[3] reported the relative incidence of caries in the first, second and third lower right molars of rats. However, as far as the writers know, this is the first report of the loss of molars from various positions in the jaw of the rat during a longevity study.

Chawla and Glickman[4] reported that protein deprivation resulted in degeneration of connective tissue of the gingiva and periodontal membrane, osteoporosis of the alveolar bone, and retardation in the deposition of the cementum. Bavetta and Bernick[5] observed that lysine deficiency caused degenerative changes in the mandibular condyle of rats, and McClure and Folk[6] found that heat-treated milk powders deficient in lysine were responsible for a high incidence of caries in rats. Chlortetracycline* was demonstrated by Stephan, Fitzgerald, McClure, Harris, and Jordan[7] to decrease caries incidence in rats. In the present study rats were fed four different levels of corn–soybean meal protein, with and without the antibiotic.

EXPERIMENTAL METHODS

Weanling rats of the Long-Evans strain were divided into 8 dietary groups, each group consisting of 6 males and 6 females. Littermates were divided among the various dietary groups. The rats were fed diets that contained four different levels of protein (11, 14, 17, and 20 per cent), each protein level with and without chlortetracycline (22 p.p.m.). The diets consisted of ground corn (45 per cent of which was held on a 20-mesh and 14 per cent on a 30-mesh screen), soybean meal, alfalfa meal (5 per cent), bone meal (0.5 per cent), limestone (1.0 per cent), salt–trace-element mineral mix (0.53 per cent), and supplementary vitamins. Calculated protein levels of 11, 14, 17, and 20 per cent were obtained by making corn meal to soybean meal percentage ratios of 86 to 7, 78 to 15, 69 to 24, and 61 to 32, respectively. The salt–trace-element mixture consisted of the following substances in grams per kg. diet: NaCl, 939.6; KI,

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* Aureomycin, Lederle Laboratories.

0.09; $MnSO_4 \cdot H_2O$, 38.2; $FeSO_4 \cdot 7H_2O$, 16.5; $CuSO_4 \cdot 5H_2O$, 5.2; and $CoCO_3$, 0.4. The following vitamins were added in mg. per kg. diet: thiamin, 22; riboflavin, 5; niacin, 50; pantothenic acid, 22; pyridoxine, 8; choline, 4,280; folic acid, 0.5; B_{12}, 0.02. Vitamins A and D were given orally in 0.1 ml. of oleum percomorpheum per week. The rats and their parents for at least three generations were supplied with city water that was fluorinated to the level of 1 p.p.m. of fluorine. The feeding was ad libitum, and each cage contained two rats. When the rats succumbed, they were decapitated, the heads autoclaved, flesh removed, and observations were made for attrition and loss of teeth. Statistical analysis of the data was made in accordance with Snedecor.[8]

RESULTS

The average number of days of survival for each of the dietary groups of rats are presented in Figure 1. The protein level and chlortetracycline had no significant influence

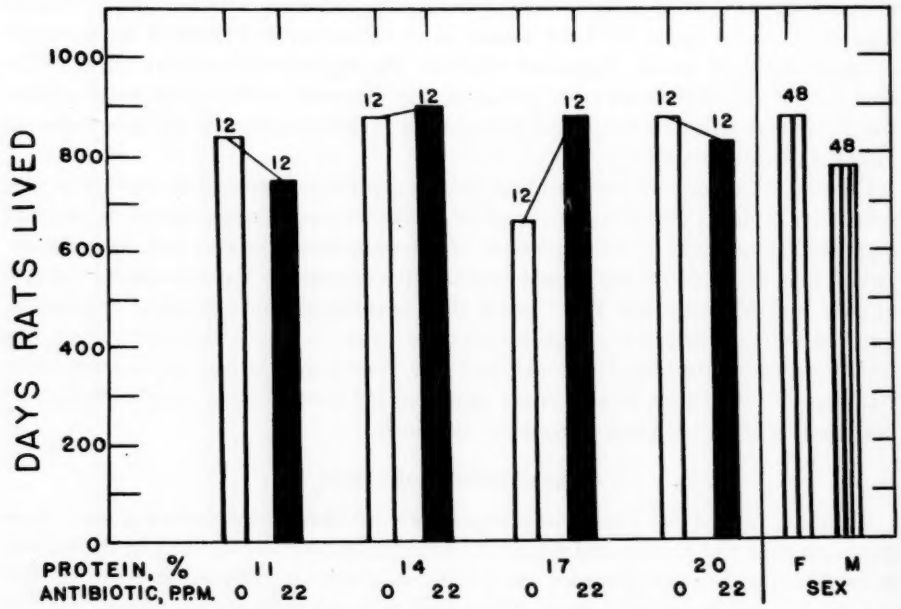

FIG. 1.—Effect of dietary protein level, chlortetracycline, and sex on survival of rats. Each bar gives the average life-span in days for the number of rats indicated at the top of the bar.

on the survival of the rats. The greater length of time of survival of the female rats was not significant. After 6 weeks on the diets the females and males fed the 11 per cent protein diets weighed 173 ± 12 and 211 ± 22 gm., respectively, while the corresponding females and males fed the 14, 17, and 20 per cent protein diets weighed 181 ± 11 and 233 ± 20 gm. ($P < 0.01$). After 7 months on the diets, the weights of the rats in the various dietary groups were equivalent statistically. The antibiotic had no effect on the growth of the rats.

After 20 months on the diets, unaided visual observations were made for caries.

Caries were scored according to Sognnaes,[3] and the caries incidence and score data are presented in Table 1. The difference in incidence of caries between the dietary groups did not quite reach significance. However, the 11 per cent dietary protein group had a smaller ($P < 0.05$) score than did the other three dietary groups. The magnitude and difference in caries scores between the four dietary groups were so small that the writers believe that little importance should be attached to them as far as any beneficial effect of one diet over the other diets is concerned. This seems to be a likely conclusion, as apparently there was no relation of caries at this time to lost molars at the time the rats died. This strain of rats is quite resistant to caries, and the few carious lesions observed were the occlusal fissure type and mostly present in the first and second lower molars.

TABLE 1*

EFFECT OF 11, 14, 17, AND 21 PER CENT PROTEIN ON CARIES INCIDENCE AND SCORE IN RATS AFTER 20 MONTHS FROM WEANING ON DIETS

Per Cent Protein	No. Rats	Caries Incidence			Caries Score		
		Av.	t	$P<0.05$	Av.	t	$P<0.05$
11.....	18	0.38	1.81	No	0.77	4.6	Yes
14.....	22	1.10	1.78	No	1.69	2.1	Yes
17.....	18	0.95	2.09	No	1.22	4.95	Yes
21.....	22	1.09			1.72		

* Scoring was made according to Sognnaes (see Ref. 3) and statistics according to Snedecor (see Ref. 8, p. 98).

By the time the rats succumbed, attrition of the teeth was quite severe, but the dietary protein level and the antibiotic had no significant effect on the extent of attrition. However, at this time numerous molars were missing. The 20 per cent dietary protein group had 11 per cent of the molars missing, compared with 13, 18, and 13 per cent for the 17, 14, and 11 per cent protein groups, respectively. The greater losses that occurred with the lower levels of protein were not significant. The corn–soybean meal in the 17 per cent diet gave calculated levels of lysine, tryptophane, phenylalanine, and valine that just met the requirements of the rat for growth.[9] The rats that received the chlortetracycline had a loss of 13 per cent of their molars compared with 14 per cent for those deprived of the antibiotic.

Data obtained for the effect of position on the loss of molars are presented in Figure 2. There was a loss of 17 per cent maxillary compared with 11 per cent of the mandibular molars, due largely to the extensive loss of the first maxillary molar ($P < 0.01$). The pattern of losing the molars was quite different between the two jaws. In the mandible 6, 10, and 17 per cent of the molars were lost in the first, second, and third positions, respectively, while in the maxilla 29, 10, and 11 per cent losses occurred in these three corresponding positions ($P < 0.01$). Fifteen per cent of the molars were lost in the left compared with a 13 per cent loss in the right quadrants.

DISCUSSION

The positions from which the molars were lost do not follow observed caries patterns. Hunt and Hoppert[1] found in their resistant strain of rats that 28.7, 37.5, and 0.04 per cent of the carious lesions occurred in the first, second, and third lower molars, with 22.5 and 11.0 per cent between the first and second and between the second and third molars, respectively. Obviously, these values have no correlation to the 6, 10, and 17 per cent losses of the first, second, and third lower molars of the present study. Sognnaes[3] observed excessive caries occurring first in the lower second molar and

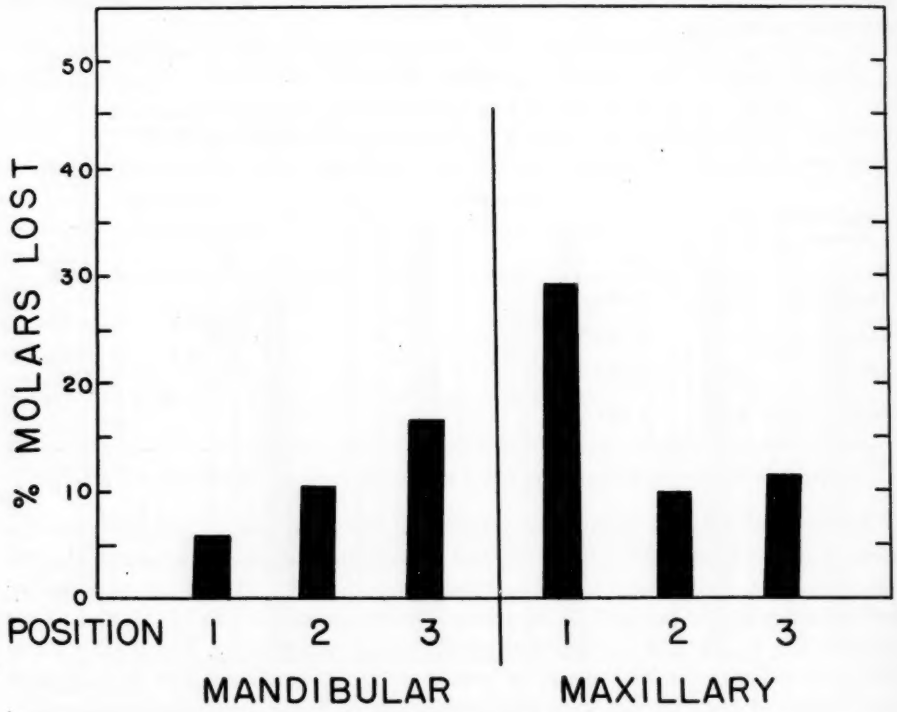

FIG. 2.—Effect of position of molars in jaw on number lost

then in the first molar and finally in the third molar. Shaw *et al.*[2] observed, in the upper jaw of the cotton rat, the least caries in the first molar, which is the position in which the greatest loss occurred in the present study. This may indicate that this molar has the least chance for the fracture-induced type of lesion and, because of less use, does not develop the capacity to withstand the gravitational and other forces that aid in the loss of teeth. However, the cotton rat was observed by Shaw *et al.*[2] to have 47, 84, and 92 per cent of the susceptible regions of the bottom first, second, and third molars to have developed caries which parallels quite closely the 6, 12, and 17 per cent losses of the lower first, second, and third molars, respectively, of the present study. Nevertheless, the Long-Evans strain of Piebald rats of this study should be more logically compared with the white rat strains of Hunt and Hoppert[1]

and Sognnaes[3] rather than the cotton rat of Shaw *et al.*,[2] even though the lost molar pattern of the lower jaw of the present study more closely follows the caries pattern of the corresponding molars of the cotton rat. The present writers do not believe that caries incidence had any dominating relation to the loss of molars in this study. Such a relationship might become effective in a strain of caries-suspectible rats. This needs to be demonstrated.

The rats of the present study appeared healthy throughout their life-span, and no evidence of periodontal disease or malformation was observed. The low level of chlortetracycline (22 p.p.m.) in these diets was much lower than the 500 p.p.m. used by Stephan *et al.*,[7] and no significant effect of the antibiotic was observed, although it tended to decrease caries and loss of teeth.

While the 11 and 14 per cent dietary protein level was lower than the requirements for good growth of young rats, it was far superior to the protein-free diets of Chawla and Glickman,[4] which contained no protein. Their diet resulted in pronounced aberrations of the gingival connective tissue, periodontal membrane, cementum, and alveolar bone. Thus the different levels of dietary protein in the present study were not low enough to demonstrate an effect on caries and loss of molars.

SUMMARY

In a longevity study with 96 rats subdivided equally into dietary groups and fed four different levels of corn–soybean meal protein diets (11, 14, 17, and 20 per cent protein), with and without chlortetracycline (22 p.p.m.), observations made when the rats succumbed showed that (*a*) 17 per cent of the maxillary, compared with 11 per cent of the mandibular, molars were missing ($P < 0.01$); (*b*) in the mandible 6, 10, and 17 per cent of the molars in the first, second, and third positions were lost, respectively, while in the maxilla 29, 10, and 11 per cent losses had occurred in these three corresponding positions ($P < 0.01$); (*c*) 15 per cent of the molars were lost in the left compared with 13 per cent in the right quadrants; and (*d*) dietary protein level and chlortetracycline had no significant effect on the number of molars lost.

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Fate of Homologous and Isologous Fresh and Stored Bone Transplanted to the Anterior Eye Chambers of Inbred Mice

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In recent years, claims have been made that bone stored in heterologous plasma remains viable and can be successfully transplanted to similar heterologous hosts. Tucker[1] has claimed that bone stored in plasma of a heterologous species maintained its viability for long periods of time. Furthermore, the storage in plasma tended to decrease the tissue specificity of the bone, making possible the direct incorporation of this type of graft into a similar heterologous host. Apparently, this possibility led Tucker to the development of a "cultured" calf bone for surgical repair of bone defects in man.

Bell[2] expressed the opinion that "healing frequently takes place in spite of what is implanted, and not necessarily because of what is used." His use of cultured calf bone in filling oral defects led him to the obvious conclusion that many of the so-called good clinical reports were based on short-term clinical and roentgenologic evaluation. It should be added that further study of a larger number of cases for longer periods of time must be made in order to derive any definite conclusions.

Fisher and Clayton[3] and Clayton[4] support the hypothesis suggested by Tucker that bone of one species can be made compatible for transplantation purposes by storing the bone in plasma from a recipient of another species. This method of soaking tissue in homologous plasma (or tissue fluid) to remove histo-incompatible factors is one of the principal controversies regarding transplantation of homologous bone grafts. Whether the graft itself actually produces new bone or becomes incorporated into the transplant site as a scaffold is one of the primary questions. Further investigation must be undertaken on a firmer foundation based on isologous and homologous transplants to non-osseous sites.

On the premise that soaking tissue in homologous tissue fluid can possibly remove the histo-incompatible features, thus allowing for transplantation to a like homologous recipient, a series of experiments were designed. The purpose of these experiments was not to duplicate exactly the conditions set up by previous investigators but to evaluate the response of homologous and isologous fresh and stored bone transplanted to the anterior eye chambers of inbred mice. This site would also allow for constant observation of the transplant.

MATERIALS AND METHODS

Female mice of the Strong A and Balb/c strains, approximately 3 months of age, were selected as experimental animals. Four groups from the two strains were estab-

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lished to be recipients of immediate (fresh, not stored) bone transplants. Immediate homotransplant groups contained 30 animals each (groups A and B); immediate isotransplant groups contained 18 animals each (groups C and D). The animals were divided equally within each of the groups to receive transplants of femoral (epiphysis and diaphysis) and parietal bone.

A donor animal of average size was sacrificed by cervical dislocation and skinned. Distal halves of femurs and medial peripheral portions of parietals were selected to represent endochondral and intramembranous bone. These portions of bone were selected because previous observations of bones from alizarin-injected animals of the same strain, sex, and approximate age showed greater alizarin deposition in the distal half of femurs, especially the epiphysis and the medial periphery of the parietals. Using aseptic procedures, the muscles were quickly removed and the bones placed in Tyrode's solution. Fragments of these pieces of bone were then cut so as to fit into a 20-gauge trocar and implanted in the anterior eye chamber of the recipient animals, using the technique described by Browning.[5, 6]

Tissue fluid was prepared for the storage of bone that would later be transplanted. Two strains of mice, 3 months old, one consisting of 12 Strong A's (average weight 18 gm.) and the other of 11 Balb/c's (average weight 23 gm.), were sacrificed. They were skinned, eviscerated, and decapitated, with a subsequent average weight of 10.5 gm. per carcass for both strains. Animals of each strain were minced with 25 cc. of sterile normal saline for 1½ minutes in a prechilled tissue homogenizer* bowl and then centrifuged at 2,400 rpm for 15 minutes. The centrifuged material separated into three layers, a top layer of fat, a middle layer of reddish-brown tissue fluid, and a bottom layer of residue. The top layer was held back, while the middle layer was decanted. Penicillin† was added to this raw tissue fluid in a ratio of 1 cc. per 100 cc. of fluid, or approximately 50,000 units per cc. of fluid. This solution was then passed through a filter,‡ and approximately 50 cc. of purified filtrate were obtained. Phenol red§ as a contaminate indicator was added to this filtrate in a ratio of 0.12 ml. per liter plus ⅛ ml. per liter. (It has been observed that in solutions such as the one described, the addition of ⅛ ml. per liter is helpful in overcoming the masking effect of the color of the tissue fluid.) At this point, the fragments of femurs and parietals (approximately ¼–½ sq. mm.), from 3-month-old donor animals, were placed in small vials and covered with 1–2 cc. of the prepared tissue fluid. These vials were then stored at 5° C.

After 40 days the vials were removed from refrigeration, and the stored bone fragments were washed in a saline solution prior to implantation. Recipient animals were 3 months of age when they received the stored transplants.

Four groups of animals were prepared to receive homotransplants of bone stored in homologous and isologous tissue fluid. Groups E and F contained 60 Balb/c strain mice designated to receive Strong A strain bone which had been stored in either Balb/c (group E) or Strong A (group F) strain tissue fluid. Groups G and H con-

* Oster Blender.

† Penicillin G Potassium (buffered with sodium citrate) aqueous solution, E. R. Squibb & Sons.

‡ Seitz type SL-3, 60 mm., fine.

§ Phenol Red, 0.5 gm/100 ml, m/10 NaOH 15.0 ml., Microbiological Associates, Bethesda, Maryland.

tained 66 Strong A strain mice designated to receive Balb/c strain bone which had been stored in either Strong A (group G) or Balb/c (group H) strain tissue fluid.

Two groups of animals were prepared to receive isotransplants of bone stored in isologous tissue fluid. Group I contained 34 Balb/c mice and Group J contained 30 Strong A mice. Fragments of stored bone were prepared and implanted in the anterior eye chambers as described previously.

Each week after transplantation, all eyes were examined under a dissecting microscope. Photographic records were also taken.[7]

Activity of the intraocular transplants was evaluated by the presence or absence of vascularization and/or uptake of alizarin red S.[8]

Because of previous experience in which alizarin was observed to be somewhat toxic to mice when administered in Tyrode's solution, it was decided to use a 1 per cent solution of alizarin in an 0.9 per cent saline solution. A difference in toxicity according to strain was also observed. Mice of the Strong A strain seemed to be more tolerant to injections of the alizarin red S solution than animals of the Balb/c strain. On the basis of these experiences, randomly selected animals from each group were injected with alizarin red S, but the remaining animals of each group were also observed periodically.

Animals used in groups A through D were given the first of three injections of alizarin red S on the thirty-sixth day after the implanting procedure. Animals used in groups E through J were injected on the twenty-first day after implantation. After three alizarin injections, the transplants were examined for staining of new bone. These animals were selected for injection at random as representative of each of the groups. The conclusions relating to alizarin uptake have been based on observations of these injected animals, the assumption being that they were representative of similar animals in their respective groups.

RESULTS

All bone fragments showed signs of vascularization in both homotransplants and isotransplants. A few of the eyes developed minor infections due to the transplantation, but, by about the third week, most of the infections had subsided.

Unstored transplants.—The pattern of activity of the homotransplants was similar to that in the isotransplants up to the third week. However, at that time, the similarity ended, and the vascularity of the transplants began to disappear, and, except for 10 of 48 transplants (21 per cent), they were soon left without a blood supply. From this time until the termination of the experiment 51 days later, no particular change was noted in either growth or resorption. During this interval the fragments appeared to change color slightly, taking on a more yellowish hue, indicating death of the transplant.

As mentioned previously, after the third week, similarity in the activity of the transplants ended, and in 16 of 32 isotransplants (50 per cent) the vascularity remained. In some of the transplants the blood vessels seemed to enter the transplanted bone itself (Fig. 1), whereas in other cases the vascular destination could not be seen or disappeared under the transplant (Fig. 6). Except for the few transitorily infected eyes, all the bone fragments appeared nearly equal in their activity. They remained the same color as at the time of transplantation, but, in 14 of 32 transplants (43.75

per cent), small areas of a lighter white material began to be manifested (Figs. 2 and 3). This material later proved to be new bone tissue, as evidenced by its absorption of alizarin (Figs. 4 and 5).

Stored bone transplants.—Bone fragments which had been stored and then transplanted showed patterns similar to those of the bone fragments of unstored homologous transplants. Thirty-six days after implantation, 34 out of a total of 144 transplants (22 per cent) showed vascularity. Of the homologous transplants of stored bone, 26 out of 96 transplants (27.5 per cent) showed vascularity, thus differing

FIG. 1.—Experiment A, immediate transplant of a diaphysis fragment 14 days after implantation. This transplant and its visible vascular supply are exemplary of 10 of 48 observed for 51 days in experiments A and B. (Orig. mag. ×45.)

slightly from the immediate homologous group wherein 10 out of 48 transplants (21 per cent) were vascularized. (It should be added that this small difference between the immediate and stored homotransplants could be attributed to the difference in time after transplantation that final observations were made.)

Of the 144 transplants of stored bone, 36 animals, representing 72 transplants, were alizarin-injected. None of the transplants, vascularized or not, indicated alizarin uptake. In 4 transplants (groups F and I, Figs. 7 and 8), large, cloudy, white outgrowths appeared, seemingly from the transplant. These outgrowths closely resembled the new bone growth in some of the immediate isotransplants but were less dense, and alizarin uptake did not occur.

DISCUSSION

Tucker[1] claims that calf osteocytes remain viable up to 28 months when stored in human plasma at 5° C. He based this claim on histologic studies and bone tissue cultures. He implies that no particular reaction was in evidence after transplantation of this treated bone, thus showing that tissue specificity was no longer a factor in the success of the graft.

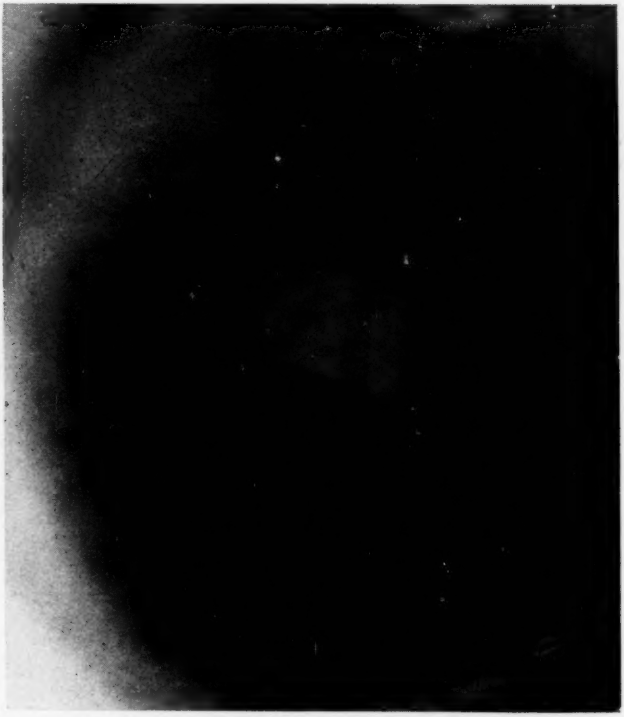

FIG. 2.—Experiment C, typical immediate transplant of an epiphysis fragment 51 days after implantation. The white cloudy extension in the eleven o'clock position picked up alizarin, suggesting new osseous tissue. This transplant was vascularized, though not visible in this picture. (Orig. mag. ×45.)

Bell[4] has stated that "healing of a graft frequently takes place in spite of what is implanted, and not necessarily because of what is used." In view of our findings, his conjecture may be well based. It has been shown by Urist and McLean[9] that bone will grow when autologously transplanted to the anterior eye chamber. Our findings corroborate their conclusions. As would be expected, the histo-incompatibility of the fresh homologous implants was apparently responsible for their failure to grow and/or persist in certain cases.

In all cases of growth (alizarin uptake and/or vascularization), the new bone was opaque and grew in a layer over the surface of the implant or in its periphery. No activity of the stored-bone transplants, except that of the 4 mentioned before (groups F and I, Figs. 7 and 8), was apparent. The white outgrowths were translucent and

FIG. 3.—Experiment C, immediate transplant of a parietal fragment 51 days after implantation. The transplant lost its vascular supply but later incorporated alizarin in its substance, especially in the rounded portion in the four o'clock position. (Orig. mag. ×45.)

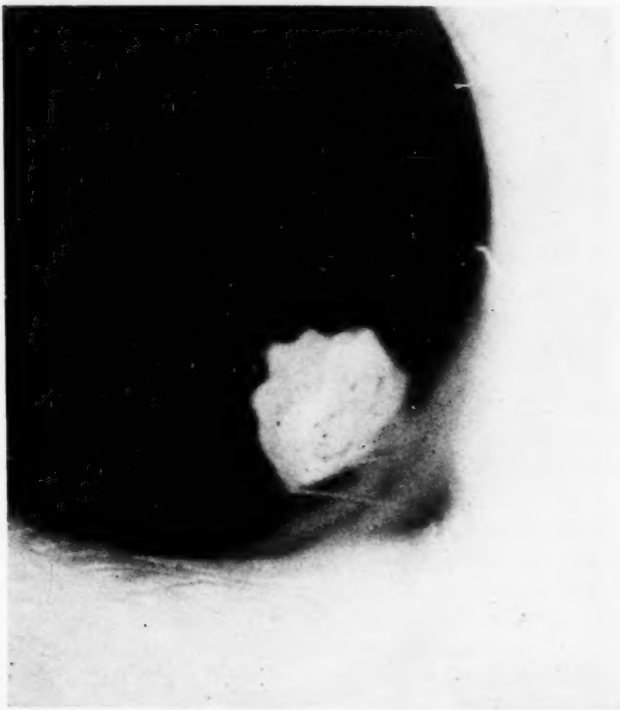

FIG. 4.—Experiment D, typical immediate transplant of an epiphysis fragment 14 days after implantation. Alizarin was incorporated on the periphery of the transplant, the greatest concentration on the quadrant nearest the pupil. (Orig. mag. ×45.)

FIG. 5.—Experiment D, immediate transplant of an epiphysis fragment 51 days after implantation. The transplant exhibited vascularization and alizarin uptake. (Orig. mag. ×45.)

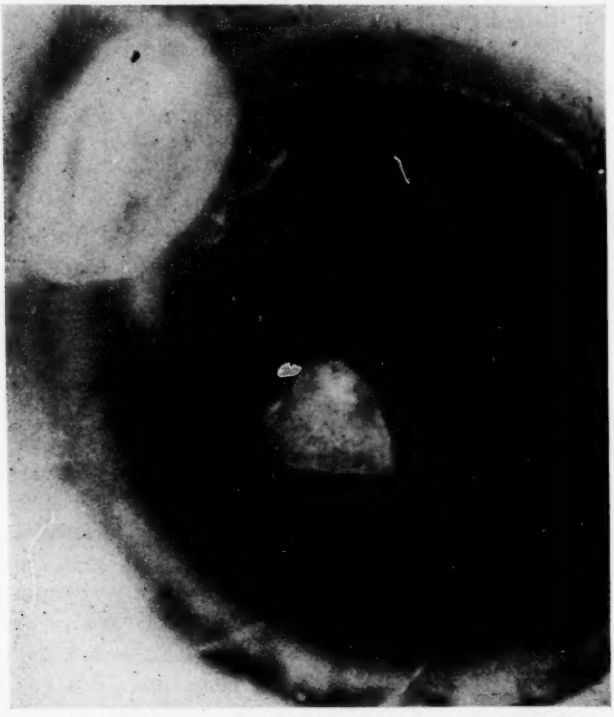

FIG. 6.—Experiment D, immediate transplant of an epiphysis fragment 51 days after implantation. The transplant lost its vascular supply but later incorporated alizarin in its substance, especially on its periphery. (Orig. mag. ×45.)

varied considerably in distance from the bone fragment, some spreading in a thin film as far away as the total diameter of the whole transplant fragment (approximately ½ mm.). Not one of these 4 white outgrowths was vascularized, nor did they show any sign of alizarin absorption; but, as evidenced by some transplants (groups C and D), vascularity of the bone fragment was not essential for alizarin uptake (Figs. 3 and 6). The fact that alizarin was not taken up suggests that the cloudy white outgrowths were not new bone growth.

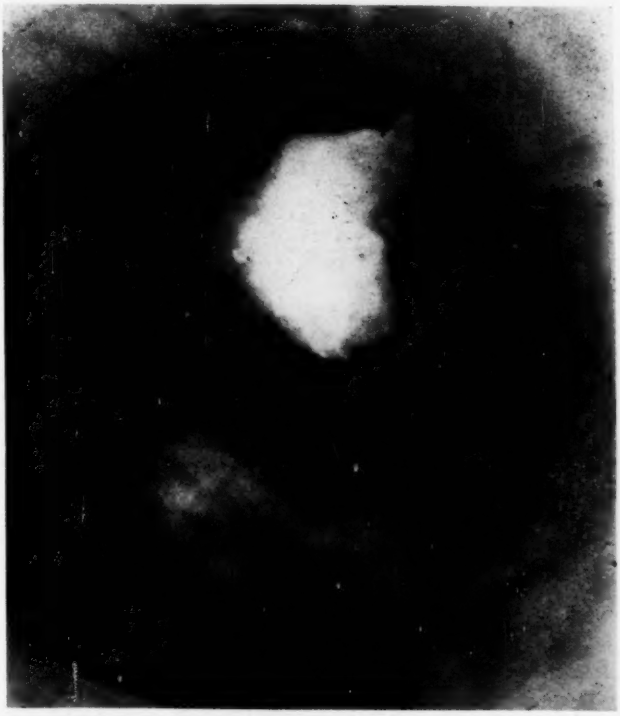

FIG. 7.—Experiment H, stored transplant of an epiphysis fragment 36 days after implantation. This transplant lost its vascular supply but developed a white cloudy outgrowth, while the transplant in the other eye retained its vascular supply. Alizarin uptake was not visible in the white cloudy growth or either transplant. (Orig. mag. ×45.)

The inactivity of these stored fragments may have been due to the storage medium. Other investigators had always used plasma rather than the tissue fluid which was substituted. However, it was felt that tissue fluid offered an environment similar to plasma for the bone storage, and it was more easily obtained.

Care was taken to keep the tissue fluid and bone fragments as sterile as possible. No special precautions were taken to check the sterility, however, as was done by Tucker. (He cultured the plasma 1 week after storage and then every 2 weeks thereafter, discarding contaminated containers.) In no instance was there a phenol red change that would indicate that our tissue fluid was contaminated.

Vascularity of some degree was associated with all transplants during their initial

phases. Because of the static nature of the bone fragments, it was necessary to have some means other than the presence or absence of vascularity to determine the activity of the transplant. Incorporation of alizarin into the bone transplant seems to be one method of assessing the life of the transplant. It should be re-emphasized that some life apparently remained in the transplant, even though vascularization might have disappeared.

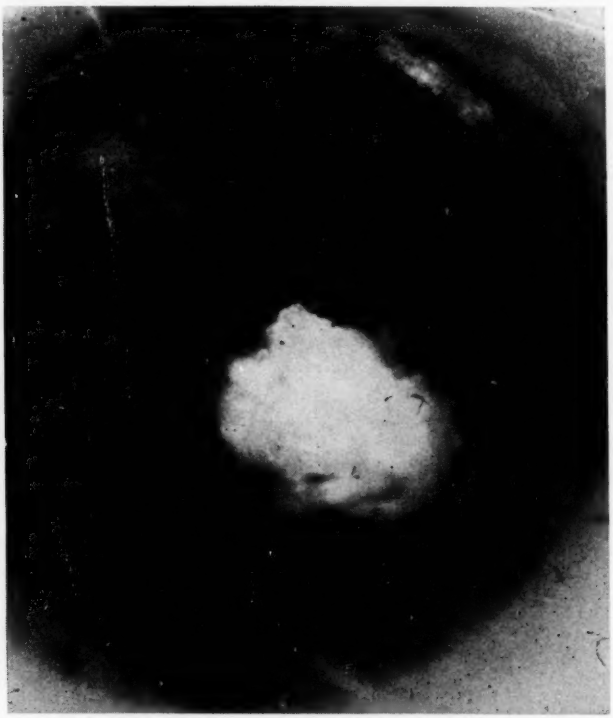

FIG. 8.—Experiment F, stored transplant of an epihysis fragment 36 days after implantation. This transplant lost its vascular supply, developed white cloudy outgrowths observed as a peripheral "rounding-off," suggesting earlier osseous deposition. Alizarin uptake was not discernible. (Orig. mag. ×45.)

It would seem that if storage of tissue fragments (in this instance, bone) in isologous or homologous tissue fluid sustains the viability of the tissue for successful transplantation, unsuccessful transplantation can result from several causes. The site where the transplant is made is an important factor in determining true viability. If this new site is a bony area in the host, it is very possible that the transplant is merely a scaffold on which the host's own bone is growing. However, if the new site of the transplant is an area not associated with the host's bone but offers the transplant a favorable environment in which growth of the transplant does occur, then there is reason for credence in this hypothesis of transferred viability.

Technique of storage, whether affecting the transplant or not, is an unanswered

question at this time. Modification of storage procedures and, similarly, variation in the recipient's body chemistry (Wiswell and Browning[10]) are being evaluated further.

SUMMARY

Without the procedure of prior storage, homologous and isologous bone fragments were transplanted directly to the anterior eye chambers of mice of Strong A and Balb/c strains and observed for 51 days. Similar bone fragments were stored in tissue fluid (both homologous and isologous) for 40 days at 5° C. and then transplanted to the anterior eye chambers of mice of Strong A and Balb/c strains and observed for 36 days.

The immediate isologous transplants developed a visible blood supply. The absorption of alizarin red S indicated the growth of new bone. There was also uptake in some non-vascularized transplants in animals injected with alizarin.

No visible absorption of alizarin red S occurred in the transplants using the stored-bone fragments, indicating no bone-forming activity. We are basing our conclusions upon the fact that alizarin red S is a vital stain that may be used in determining the growth of bone in non-osseous transplantation sites.

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Tubular Enamel in Some Sea Fishes in Southern Africa

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Tubular enamel has been described in fishes occurring in the Northern Atlantic by Aitchison,[1] but, as far as can be ascertained, it has not been described before in any sea fishes in Southern Africa.

MATERIALS AND METHODS

The enamel of the posterior molariform teeth of five fishes of the family Sparidae (Smith[2]) was studied: (1) *Chrysoblephus laticeps,* commonly known as the "red roman"; (2) *Chrysoblephus gibbiceps,* commonly known as "red stumpnose"; (3) *Chrysoblephus cristiceps,* commonly known as "dageraad"; (4) *Cymatoceps nasutus,* commonly known as "musselcracker," whose posterior molariform teeth differ from *Sparodon durbanensis;* and (5) *Pterogymnus laniarius,* known as "panga." These fishes are usually found among the rocks along the coast of Southern Africa. They live mostly on rock mussels. Their anterior teeth are sharp for tearing the mussels from the rocks and then crushing them with their molariform posterior teeth.

Ground sections of the posterior teeth of the above-mentioned fishes were examined. *Chrysoblephus laticeps* has one row of large conical posterior molariform teeth and a number of small cone-shaped molariform teeth lingual to the large ones (Fig. 1). The posterior teeth of *Chrysoblephus gibbiceps* are dome-shaped and placed similarly to those of *Chrysoblephus laticeps* (Fig. 2).

Chrysoblephus cristiceps has a row of posterior buccal molariform teeth, the mesial half of which is conical, while the distal half is dome-shaped. Lingual to this row are numerous small, irregularly placed, dome-shaped teeth (Fig. 3). The posterior teeth of *Cymatoceps nasutus* consist of a single row of large, dome-shaped molariform teeth buccally and numerous smaller similar teeth situated lingually to this row (Fig. 4). *Pterogymnus laniarius* has two rows of posterior molariform teeth. The four mesial teeth of the buccal row are conical, while the distal molariform teeth are dome-shaped. The lingual row consists of dome-shaped molariform teeth (Fig. 5).

RESULTS

Figure 6 shows the tubular enamel of *Chrysoblephus laticeps*. The tubes penetrate the enamel from the periphery only. A small area adjoining the dentino-enamel junction contains no tubules. The same structure is found in *C. gibbiceps* (Fig. 7). Figure 8 shows the same tubular enamel in *C. cristiceps*.

The tubular enamel in *Cymatoceps nasutus* (Fig. 9) shows that some of the tubes

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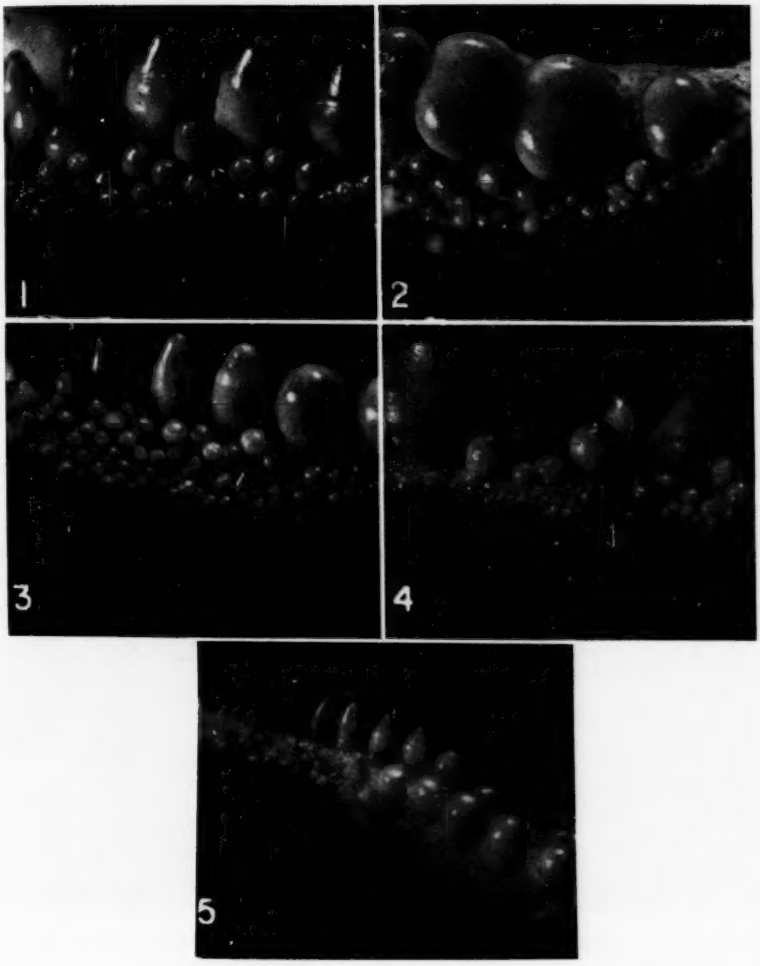

FIG. 1.—Posterior teeth of *Chrysoblephus laticeps*
FIG. 2.—Posterior teeth of *Chrysoblephus gibbiceps*
FIG. 3.—Posterior teeth of *Chrysoblephus cristiceps*
FIG. 4.—Posterior teeth of *Cymatoceps nasutus*
FIG. 5.—Posterior teeth of *Pterogymnus laniarius*

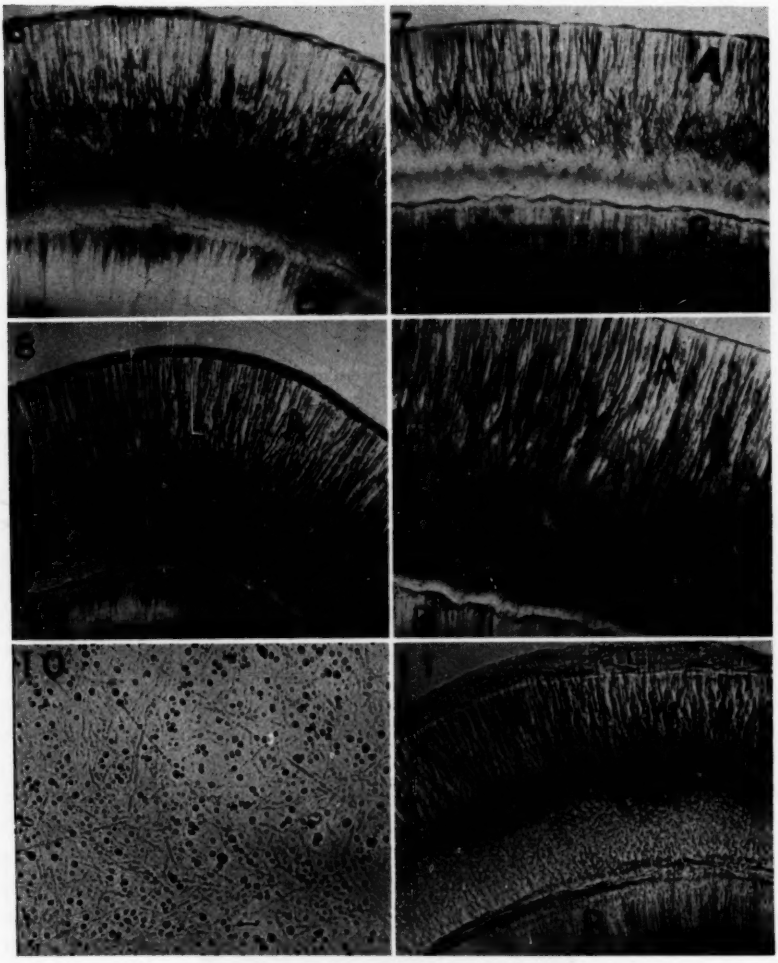

FIG. 6.—Tranverse section of *Chrysoblephus laticeps*, showing *A*, tubules open to the periphery; *B*, dentin. (Mag. ×80.)

FIG. 7.—Transverse section of *Chrysoblephus gibbiceps*, showing *A*, tubules open to the periphery; *B*, dentin. (Mag. ×80.)

FIG. 8.—Tubular enamel in *Chrysoblephus cristiceps*, showing *A*, tubules open to the periphery; *B*, dentin. (Mag. ×80.)

FIG. 9.—Tubular enamel in *Cymatoceps nasutus*, showing some tubes penetrating the entire thickness of the enamel. *A*, tubules; *B*, dentin. (Mag. ×80.)

FIG. 10.—Replica of cross-section of enamel, showing numerous tubules. (Mag. ×80.)

FIG. 11.—Tubular enamel of *Pterogymnus laniarius*, showing *A*, tubules open to the periphery; *B*, dentin. (Mag. ×80.)

penetrate the entire thickness of the enamel from the periphery to the dentin. Figure 10 is a replica of the cross-section of the enamel and shows the numerous tubules.

The tubular enamel of *Pterogymnus laniarius* is the same as that of the first three described (Fig. 11).

DISCUSSION

The only difference between the tubular enamel in the five fishes described is that some of the tubes of *Cymatoceps nasutus* penetrate the entire thickness of the enamel (Fig. 9). Aitchison[1] is of the opinion that no tube ever penetrates the entire thickness of the enamel. Scott and Symons[3] state that, among the members of the marsupial order (with the exception of the rodent-like wombat), the enamel tubules pass through the whole thickness of the enamel to the surface. The tubules of the enamel of the five fishes described appear to contain minute granules.

SUMMARY

Tubular enamel was described in five fishes of the family Sparidae, sea fishes of Southern Africa.

I wish to thank Professor C. L. de Jager, director, Oral and Dental Hospital, University of Pretoria, for his assistance and his permission to publish this article. Grateful acknowledgment is made to Mr. J. Nell for the photographs.

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Effect of Surface Alteration on the Permeability of Enamel to a Lactate Buffer

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The solution rate of enamel in acid has been studied by five techniques. The one most frequently used involves the solution rate of powdered enamel. The investigators using this have included Volker,[1] Suess and Fosdick,[2] Bibby,[3] and others.[4–6] This approach has been used widely because of its simplicity and convenience, even though it produces disorientation of histologic structures and permits acid to act upon a greatly extended enamel surface. Ideally, acid should act only upon the outer enamel surface, which is the part initially affected by dental caries.

The other four approaches make use of intact enamel structures. Some investigators have studied the change in hardness that results from the increasing porosity that is produced by decalcifying solutions.[7, 8] This approach, however, fails to disclose loss of a layer from the enamel surface and is insensitive to the development of porosity in deeper layers. At best, it appears to be a semiquantitative index of developing porosity in the outermost layer of tooth enamel.

The solution rate has also been determined by Brudevold's window technique[9] and applied by Sullivan,[10] Darling,[11] Ericsson,[12] and Crouse, Swartz, and Phillips.[13] Brudevold, Sullivan, and Darling have observed that surface enamel is more resistant to the action of acid than is internal enamel. Crouse *et al.* made alterations in the surface smoothness and determined the effect of these changes on the solution rate. Crouse was unable to observe a statistically significant difference between surfaces roughened with emery paper and polished enamel, even though his averages indicated that slightly more calcium or phosphate was removed from roughened surfaces. This approach using the rate of solution of phosphate from intact teeth (Brudevold's technique) has no inherent theoretical disadvantages but suffers from inefficiency in design of experiment. An investigator has difficulty in studying more than one variable on a tooth, in making repeated observations on the same section at different times, or in determining the exact depth to which enamel is penetrated by the acidic buffer.

The first two defects also apply to the technique used by Coolidge, Besic, and Jacobs,[14] and Besic,[15] who used the Brudevold window technique but sectioned the whole tooth, in order to observe birefringence changes in the enamel. Their method permits observation of the depth of penetration of the solutions but does not afford opportunity to obtain control pictures from which measurements and comparisons can be made.

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A technique recently developed in this laboratory[16] eliminates some of the above problem, by studying the section after it is imbedded in epoxy resin between glass slides and allowing only the outer surface to be attacked by the acid buffer. Several sections can be obtained from the same tooth and several variables studied simultaneously on one section. This approach was used to investigate the influence of abrasion and chemical treatment of the outer surface of tooth enamel, on the rate of penetration of pH 5, 1.0 molar, lactate buffer.

EXPERIMENTAL METHODS

1. *Mechanical alteration of enamel surface.*—In Study A two teeth were selected from a group of freshly extracted upper central incisors that were normal in appearance. The teeth were cleaned of debris with a dull knife. The middle three-fourths of the labial surfaces were divided into five areas, incisogingivally. The surfaces of four of these areas were altered incisogingivally by different types of abrasive devices mounted on rotary instruments. The first was abraded for 5 seconds by a rough sandpaper disk, the second by a rubber polishing wheel, the fourth by a carborundum stone, and the fifth by a diamond disk. The third segment was left untouched and served as a control. Longitudinal sections of the crown were cut and lapped to 125 μ. Six such sections were obtained from each tooth. They were mounted between glass slides in such a manner that the entire outer enamel surface was exposed. The rubber-base impression technique was employed.[16]

The second tooth was treated in exactly the same way except that the sequence of surface alterations by the different abrasive instruments was reversed.

In study B one central incisor was selected for a second study. Incisogingivally, the middle three-fourths of the labial surface was divided into three areas. The incisal third of this area was not treated, thus being used as a control. The surfaces of the two remaining gingival thirds were both roughened superficially with a diamond disk. The roughened surface of the middle third was made smooth with a fine sandpaper disk. Six longitudinal sections were prepared and treated by the methods described in study A.

2. *Influence of topical application of NaF solution to enamel surface.*—Four upper central incisors were selected for this study. The teeth were cleaned of debris with a dull knife. The mesiodistal half of the labial surface of each of the teeth was covered with adhesive tape. A 2 per cent aqueous solution of sodium fluoride was topically applied to one half of each of the teeth. The other half was covered with adhesive tape to prevent its exposure to the solution. After the solution had dried, the adhesive tape was removed. Two of the teeth were immersed in 10 ml. of fresh saliva. The remaining two teeth were immersed in 10 ml. of distilled water. The saliva and the distilled water were changed every 24 hours during the week days for a period of 3 weeks. During this procedure the teeth were removed at 1-week intervals, dried, and sodium fluoride applications were again made in the same areas (total of three applications). Six sections were obtained from each of the four teeth, three from the half of the tooth that had been left untreated, representing the control, and three from the area treated with sodium fluoride. Thus each control and variable was represented by six sections obtained from the same two teeth. Sections were then mounted and treated as described before.

3. *Influence of saliva on abraded surfaces.*—Four freshly extracted, upper central incisors were selected, the surfaces shaved with a rough sandpaper disk, and mounted on a rotary instrument. One half of each tooth was completely covered with wax. Two teeth were immersed in 20 ml. of saliva, and the other two were immersed in distilled water. Both solutions were changed on week days every 2 hours for a period of 3 weeks. After 3 weeks the wax was removed from the teeth. Six sections were obtained from each tooth, three from the control area covered with wax and three from the area treated with saliva or water. The sections were prepared, mounted, and treated in a similar fashion as that described in study A.

Transparencies* were taken initially for all mounted sections in the addition position under a polarizing microscope, using a first-order red plate and a 32-mm. objective. Three sections were immersed in one coplin jar containing 60 ml. of 1 molar lactate buffer solution at pH 5. This jar was set in an incubator at 37° C. Fresh solutions were employed after every 168 hours. Transparencies were obtained daily during week days for a period of 2 weeks. The distance of penetration in millimeters and the degree of cavitation in millimeters was obtained by projecting the transparencies at the standard magnification of 250 times and by measuring the change with a millimeter scale.

RESULTS

Readings of penetration of enamel for each of the 12 sections were obtained at different time intervals. Means of all 12 readings for penetration for the controls and for each variable have been calculated and are presented in Figure 1.

Each of the 12 sections showed a greater penetration of enamel through those surfaces that were treated with a diamond disk than through surfaces which served as controls. This indicates a statistically significant difference ($P < 0.01$).[17] The average penetration was also greater in those areas treated with stone and sandpaper disks than in the control areas, but this difference was not statistically significant. On the other hand, areas treated with rubber in all 12 sections showed less degrees of penetration than did the control areas. This difference was also statistically significant.

Six different sections were used for the study on the influence of polishing after grinding. The depth of enamel penetration has been recorded from the transparencies, and the means of the six readings each for the control and the two variables have been calculated for every time interval and are recorded graphically in Figure 2. The greatest penetration was evident in areas of enamel that had been treated with diamond disk; the least penetration was observed in control areas. Areas which were treated with a diamond disk and later altered by a fine sandpaper disk showed an intermediate amount of enamel penetration.

From the two previous studies, A and B, two inferences can be drawn. First, roughening of the enamel surface produces a considerable increase in the rate of penetration of acidic buffer; second, polishing of a roughened surface of enamel increases its resistance to decalcification. The polishing we employed did not raise the resistance of the smooth surface to equal that of the original enamel surfaces either because the polishing agent was too coarse or because the original enamel has a higher inherent resistance to acid attack.

* Kodachrome.

Topical application of NaF to enamel surface.—The amount of penetration was recorded from each of the 24 sections in this study. The mean values of penetration were obtained at each time interval for the four sets of six sections. These four sets were the controls for saliva series, sections treated with sodium fluoride and immersed in saliva, controls for water series, and sections treated with sodium fluoride and immersed in water. Average values for penetration are given in graphic form in Figure 3. Less penetration occurred initially on the sections treated with sodium fluoride than on those which served as controls. Soon thereafter, penetration rates changed order at random, and there was practically no difference after 264 hours. NaF treatment did not appear to impede the penetration of decalcification into subsurface enamel. The action of fluoride was limited to protection of the surface only.

Influence of saliva on abraded surfaces.—The amount of penetration at each time interval was recorded for the twenty-four sections employed in this series. The mean values of penetration were calculated for each of the four sets of six sections, representing the controls of saliva series, sections treated with saliva, controls of water

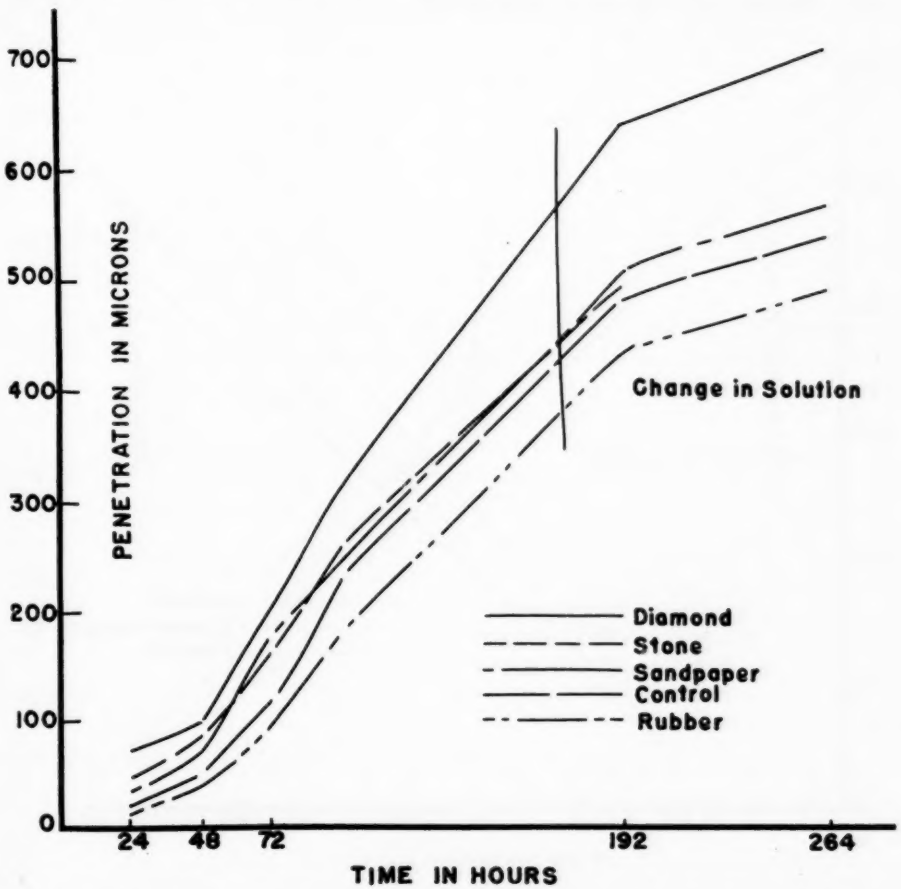

FIG. 1.—Influence of surface abrasion on the movement of the decalcification front into enamel

series, and sections treated with water. These means have been calculated at different time intervals and have been presented in a graphic form in Figure 4.

DISCUSSION

The most surprising deviation from theory is the constant rate of penetration of tooth enamel by lactate buffers, shown in Figures 1–4. It is true that there is sometimes a lag until the initial outer surface of the enamel has been penetrated, but from distances beneath the surface of 0.04–0.10 mm. the rate of penetration seems to be linear with respect to time. This finding is especially evident in Figure 2, where the greatest deviation from linearity amounts to 0.012 mm. If diffusion were limiting the rate of attack, the rate of penetration should decrease as the layer of decalcified enamel became thicker, since the diffusion paths become longer, both for buffer and for ions dissolved from the decalcification front. By Adolphe Fich's law, the diffusion velocity at distance X in a medium is inversely proportional to X, rather than independent of X. Since the penetration rate is independent of X, we can assume that diffusion does not limit the rate of penetration.

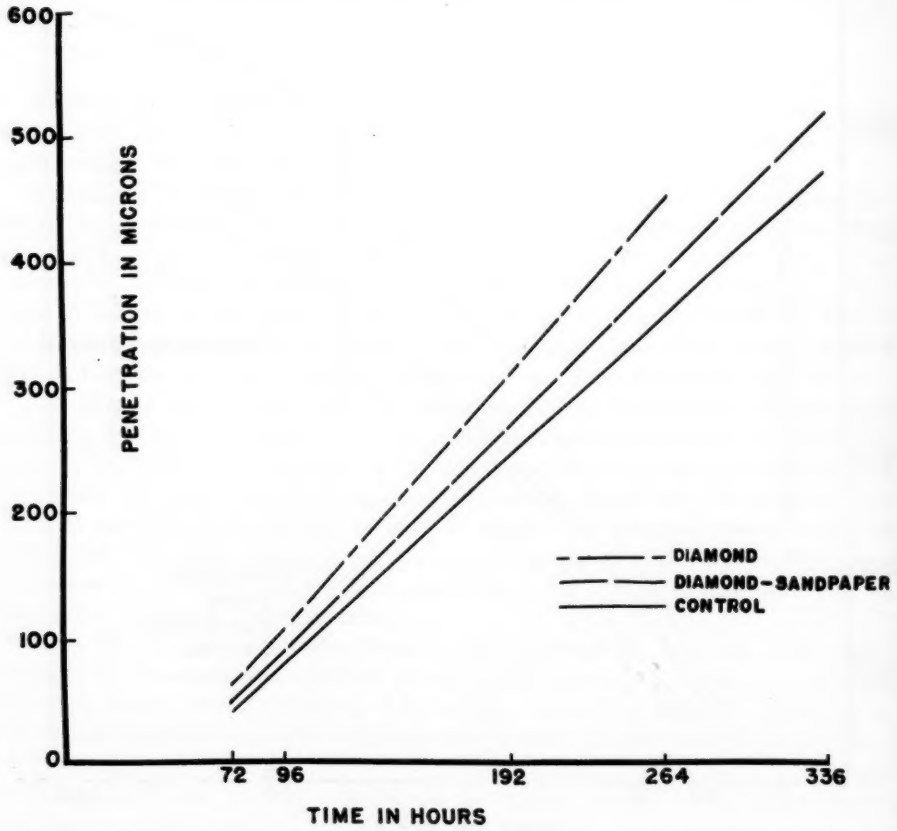

FIG. 2.—Influence of surface abrasion and polishing on the movement of decalcification front into enamel.

It is also difficult to explain why penetration should continue deep into the subsurface. The solution of lactate buffer remains in pore spaces in contact with tooth enamel for days. During this time it certainly becomes saturated with respect to calcium and phosphate, and its pH must approach neutrality. The solution that is actually within the enamel pore spaces must be essentially neutral and essentially saturated with respect to enamel. If the buffer is neutralized and saturated, why does enamel continue to dissolve?

The only explanation that can be offered at this time is based on the assumption that the enamel acts as an ion-exchange resin, in which the rate-limiting reaction is an exchange between solution and solid. The exchanging would be between the hydrogen ion for calcium, or lactate ion for carbonate or phosphate; perhaps both exchanges occur simultaneously. Ion-exchange resins are found among phosphates, and their exchange rates are known to be low, in some cases requiring several days for exchange to take place.[18]

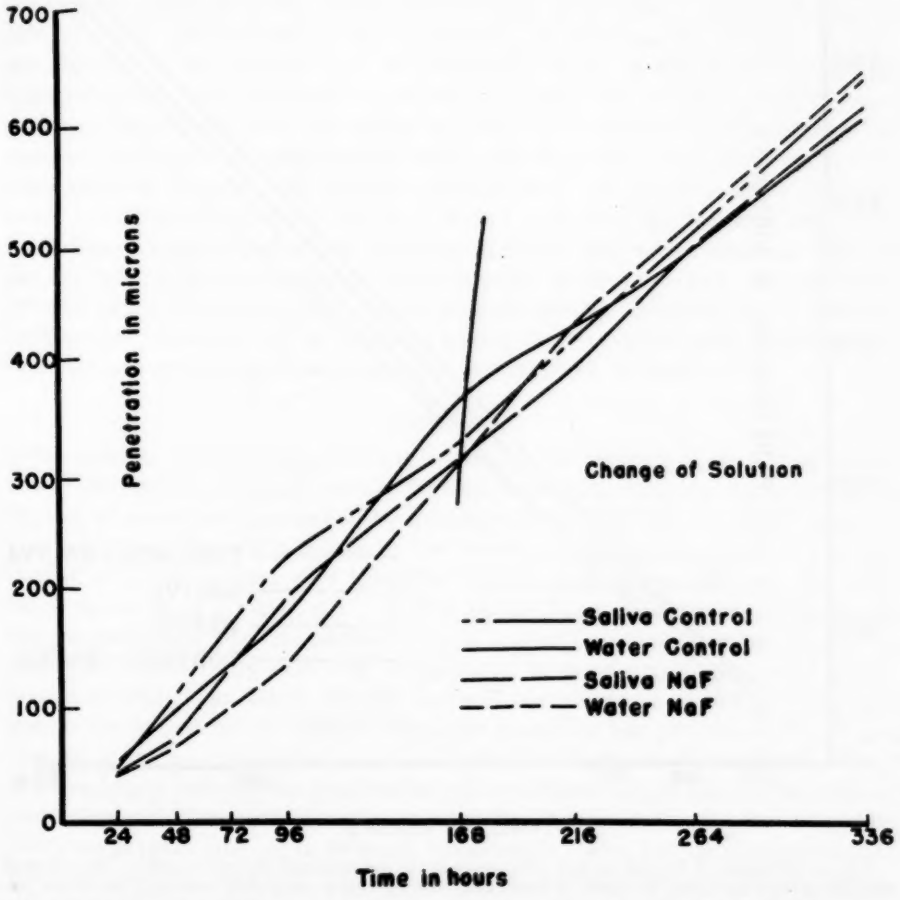

FIG. 3.—Influence of water and saliva on the penetration rate of a lactate buffer into tooth enamel. Saliva treatment causes a slight decrease and water treatment a slight increase in rate of penetration.

This concept does not disregard the dissolution of tooth enamel by acid. Dissolution occurs in case the pH is low, the buffer is strong, the buffer capacity is large, or the solution unsaturated. Dissolution removes the solid which might show exchange reactions. If conditions are such as to encourage penetration rather than cavitation, then we have a clue that the behavior of enamel parallels what is known for ion-exchange resins.

There is no indication from our work of any special resistance of the outer enamel surface. Sometimes the original enamel surface is resistant and at other times it appears to offer little resistance to penetration. The initial resistance may arise either from the smoothness of the enamel or from a deposition of salivary proteins thereon. This finding is not contradictory to the studies by Brudevold.[9] His findings are con-

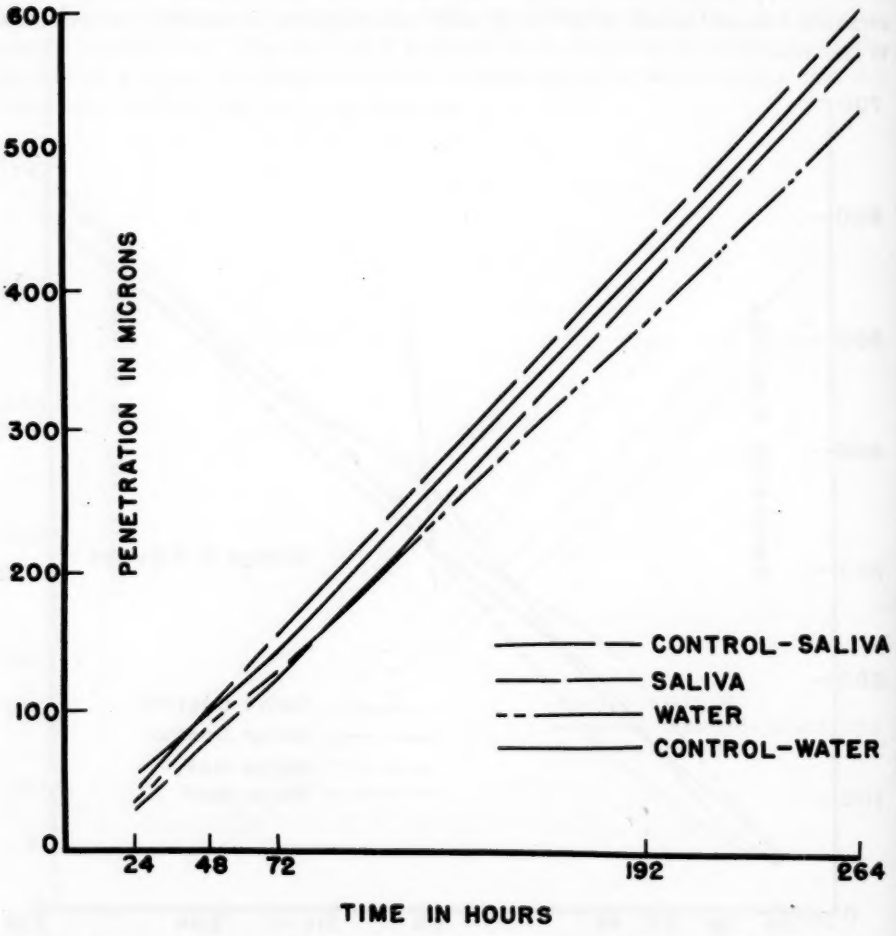

FIG. 4.—Influence of fluoride topical treatments in the presence and absence of saliva. The action of fluoride persists for about 96 hours of continuous etching and is completely lost after 264 hours' immersion in buffer.

cerned with the rate of dissolution of phosphate in a buffer that has much more solvent power than the one we employed, and he bases his criterion on the loss of mineral from enamel rather than on the rate of movement of a decalcification front. These findings have a bearing both on methodology and dental practice. Investigators studying the solubility of whole teeth or of enamel sections will, in the future, have to be concerned with the roughness or smoothness of the enamel surface. When comparisons are made between treated and untreated enamel, care must be taken to insure that the surface smoothness of the two groups is identical after treatment; if dentifrice is studied, the kind and particle size of the abrasives in the control and experimental dentifrices must be the same. These findings may also have some meaning with respect to dental practice. Abrasion of tooth enamel with coarse stones could theoretically lead to greater enamel solubility, and any mechanical action of dental instruments on tooth enamel should be followed by careful polishing of the surface.

The superficiality of the protective action resulting from topical fluoride treatment of enamel is indicated by our finding that the layer is lost after 2 days immersion in pH 5, 1 molar, lactate buffer. This superficiality of protection has bearing, at least theoretically, on the relative value of introducing fluoride in the enamel surface by different routes, such as topical treatment, dentifrice, and drinking water. Topical treatment should have much less inhibitory action on dental caries of persons having rampant caries than on dental caries among persons whose caries increment is low. When caries is rampant, the protective fluoride layer will be lost shortly after the initial topical treatment. From this time onward until the next treatment the attack rate on those surfaces undergoing rapid decalcification will not be retarded. Surfaces that are being attacked slowly may have remnants of the protective layer persisting between topical treatments. This means that the greatest influence that is possible from topical treatment will be disclosed among those patients whose caries rate is moderate and on those tooth surfaces which are less prone to dental caries.

SUMMARY

The purpose of this investigation was to evaluate the influence of mechanical alteration of the surface of enamel sections through scratching, polishing, and grinding on the rate of subsurface penetration by a lactate buffer. The effect of topical applications of sodium fluoride solutions in the presence and absence of saliva was also studied. Sixty-six sections were imbedded between glass slides with only the outer edges exposed to a 1 molar, pH 5, lactate buffer. Transparencies were taken at 32 times magnification with the section between crossed Polaroids. The degree of penetration was measured by ruler after projection of the transparencies at a total magnification of 250 times. Color changes disclosed the migration of the decalcification front to the nearest micron. Roughening of the enamel surface produced a 27 per cent increase in the rate of penetration of the buffer; polishing of the roughened surface with sandpaper reduced the penetration rate to within 10 per cent of the original penetration rate. The sections that were treated with sodium fluoride showed greater resistance to penetration up to 96 hours' immersion in buffer, but the total penetration was practically the same after 264 hours. Sections treated with saliva showed slightly less penetration than did the control which had been treated with water.

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A Quantitative Analysis of the Decrease in Width of the Mandibular Arch during Forced Movements of the Mandible

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The suggestion that the medial angulation of the external pterygoid muscles will effect a contracting action upon the two halves of the jaw was made by Grunewald in 1921.[1] More recently, De Brul and Sicher[2] demonstrated lines of stress near the symphysis of the mandible by squeezing the condyles together with finger pressure at the insertion of the external pterygoids.* They reported a distortion (in the living) between the last mandibular molars which was up to ½ mm. less when the jaw was forcefully protruded than when it was at rest. In an effort to characterize quantitatively the decrease in mandibular width from the rest position to a forced protrusive and a wide-open position the following study was undertaken.

EXPERIMENTAL METHODS

Twenty dental students were selected at random, and a stone model of the lower dentition was cast from an alginate impression. Metal splints were constructed for the stone model to fit the occlusal surface of the bicuspids and molars on each side of the arch (Fig. 1). The splints were tapped on the buccal and lingual surfaces to receive screws to hold the splints in place. All splints were drilled on the occlusal surface over the first bicuspid and second molars to receive pegs attached to a measuring device. A scissors-like measuring tool was developed, using a gauge with 5/1000-inch range (Fig. 2).

The splints were secured to the teeth on the right and left sides of the mandibular arch. Pins on the measuring device were positioned in the holes drilled over the first bicuspids. With the mandible in rest position, the gauge was zeroed.

Readings were taken when the patient opened the mouth as far as possible and when the mandible was in a forced protrusive position. The procedure was then repeated in the second-molar region. Although a minimum of three readings was taken for each position, only the maximum readings were recorded.

It was necessary to change the position of the gauge to accommodate variations in the anatomical width of the mandibular arch. This made it impractical to calibrate the instrument for all variations. A reading in millimeters was obtained by transferring directly from the measuring device by use of a micrometer.

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* Stresscoat, Magnaflux Corp., Chicago, Illinois, was employed.

RESULTS

Table 1 indicates the change occurring across the arch in the second-molar region from rest position to a wide-open position and at a forced protrusive position of the mandible. In the 20 patients studied, the average dimensional change showed a decrease in width from rest to wide open of 0.4 mm. and in protrusion of 0.5 mm. The maximum recorded change of 1.5-mm. decrease in width occurred in the forced protrusive position. Minimal dimensional changes in the bicuspid region were observable, but a quantitative analysis was unobtainable by the method used in this study.

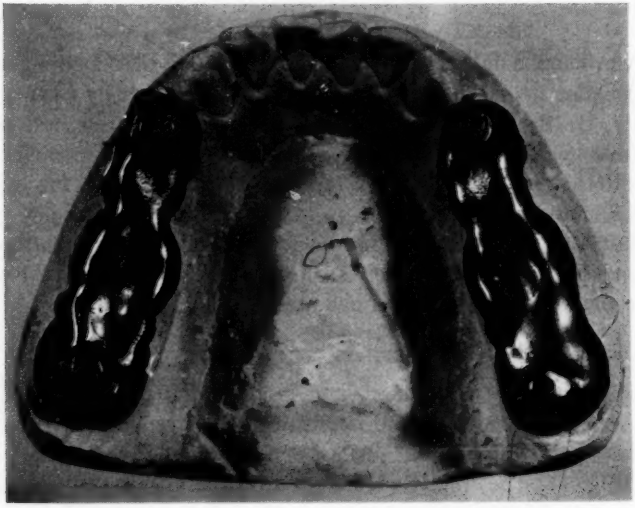

FIG. 1.—Metal splints with holes drilled parallel to receive measuring device

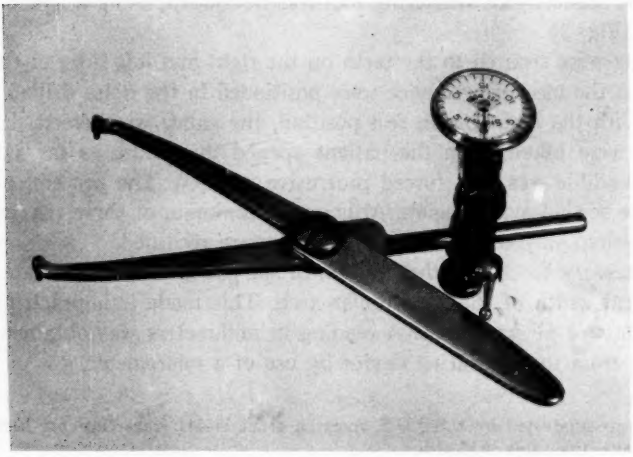

FIG. 2.—Measuring device with gauge

SUMMARY

A procedure is described for quantitatively analyzing the decrease in mandibular width occurring from rest to a forced wide-open and forced protruded position of the mandible. An analysis of the 20 patients studied indicated that the nature of the response in all cases was similar; a decrease in mandibular width in the molar region from rest to wide-open and in forced protrusion was demonstrated.

TABLE 1

DIMENSIONAL CHANGE MEASURED ACROSS MANDIBULAR ARCH IN SECOND-MOLAR REGIONS

Case No.	Wide-open (Mm.)	Protrusive (Mm.)	Case No.	Wide-open (Mm.)	Protrusive (Mm.)
1	0.3	0.7	11	0.5	0.7
2	0.5	0.3	12	0.2	0.3
3	0.2	0.3	13	0.3	0.4
4	0.3	0.4	14	0.5	0.7
5	0.2	0.3	15	0.5	0.6
6	0.3	0.2	16	0.5	0.9
7	0.5	0.5	17	1.4	1.5
8	0.3	0.4	18	0.5	0.6
9	0.5	0.6	19	0.2	0.4
10	0.5	0.6	20	0.4	0.3

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Influence of Occlusal Wear and Age on Formation of Dentin and Size of Pulp Chamber

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The phenomenon of wear—attrition, abrasion—of teeth can be observed in ancient man as well as in contemporary individuals. The most common explanation offered in the literature regarding the influence of wear on dentin and pulp is that, in response to this stimulus, nature develops a calcific deposit—the so-called secondary dentin—thus causing a recession of the pulp as the wearing process progresses. It has also been asserted that this formation takes place opposite the abraded area, i.e., between the injury and the pulp.[1–6] In addition to abrasion, other factors, such as age, have been mentioned as causing secondary dentin formation,[7–10] and it has also been suggested that this formation may even occur through a reflex action.[1, 11]

In a previous study examining ancient skeletal material of which the teeth had suffered a considerable amount of wear of varying degrees, it was observed that the age factor would probably better explain the encroachment of the pulp tissue by progressive calcification from all directions than would the functional factor of wear but that excessive wear no doubt influences the degree of calcification to some extent.[12]

The purpose of the present study was to ascertain the influence of occlusal wear, and age on the formation of dentin. The sites of dentin formation in relation to the size of the pulp chamber were also investigated, no distinction being made between primary and so-called secondary dentin. The presence of "pulp stones" was not taken into consideration.

MATERIALS AND METHODS

One hundred and sixty-eight left permanent mandibular first molars of both sexes were used for this study. The sample included 93 molars of contemporary individuals in vivo ranging from six to seventy years of age, and 75 molars belonging to ancient skeletal material *in situ,* from five to forty-nine years of age at death, the latter material extending over the time-span 3000 B.C.—A.D. 1900, i.e., from Neolithic times to the beginning of the present century. The age at death of each of the ancient skeletal specimens was taken from previous anthropological studies.[13, 14] With the criteria available at present, it is not possible to give this age to a close degree of accuracy,[15] especially where the age exceeds some twenty-five years.[16] The numbers of specimens of both groups examined are shown in Table 1 according to age.

The examination, visual and intraoral radiographic, was limited to normally aligned, sound, left, permanent, mandibular first molars, free of caries and/or any operative work. The calcification and development of this tooth in specimens belonging to our

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youngest cases were found at the stage at which the enamel cuticle was completed, formation of dentin had continued, and the floor of the pulp chamber (the cleft) at the bifurcation of the roots had begun to be formed. The gingival extremity at the bifurcation of the roots and the dentino-enamel junction at the center of the occlusal surface of this tooth were considered as two fixed points. For each case the age and estimated degree of occlusal wear were recorded in special charts, the degree of occlusal wear being assessed according to the method described in a previous paper.[12]

In the present study, only teeth with degrees of occlusal wear *0–V* inclusive (Fig. 1) were taken into consideration. Of the teeth showing wear at degree *V*, those with no traces of enamel preserved at the dentino-enamel junction at the center of the occlusal surface were excluded, since this is one of the fixed points referred to above and constitutes one of the indispensable bases of our measurements, as will be seen below. The distribution of cases in both groups according to the assessed degree of occlusal wear is shown in Table 2.

TABLE 1

MATERIAL CLASSIFIED ACCORDING TO AGE GROUPS

Age Group	No. of Cases		Cumulative				Mean Degree of Occlusal Wear	
			No. of Cases		Per Cent of Cases			
	Contemporary	Ancient Skeletal Material	Contemporary	Ancient Skeletal	Contemporary	Ancient Skeletal	Contemporary Material	Ancient Skeletal Material
5–10	9	9	9	9	10	12	0.1	0.8
11–20	19	9	28	18	30	23	0.8	2.1
21–30	19	26	47	44	50	59	1.4	2.6
31–40	12	20	59	64	63	85	2.8	3.2
41–50	16	11	75	75	80	100	1.6	3.4
51–60	9	.....	84	.....	90	.....	3.4	.....
61–70	9	.....	93	.....	100	.....	3.0	.....
Total	93	75	.....	.....	.....	.....	1.7	2.6

TABLE 2

MATERIAL CLASSIFIED ACCORDING TO DEGREE OF OCCLUSAL WEAR

Degree of Occlusal Wear	No. of Cases		Cumulative Percentage of Cases		Age Range	
	Contemporary	Ancient Skeletal	Contemporary	Ancient Skeletal	Contemporary	Ancient Skeletal
0	15	4	16	5	6–24	5– 6
I	37	7	56	15	10–53	6–28
II	18	26	75	50	12–70	9–45
III	11	19	86	75	21–60	15–45
IV	8	15	94	95	36–67	25–40
V	4	4	100	100	36–70	40–49
Total	93	75	.....	.....	6–70	5–49

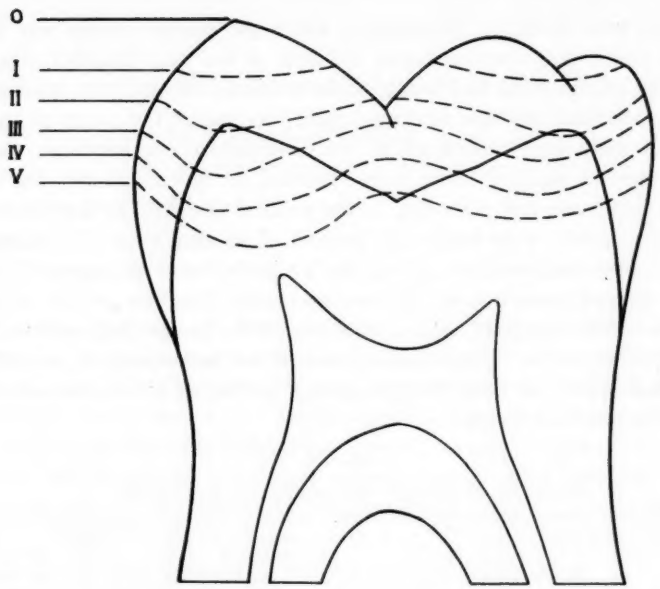

FIG. 1.—Cross-sectional anatomy from the buccal aspect of a left permanent mandibular first molar, showing successive stages of occlusal wear, from degree *0* to *V*.

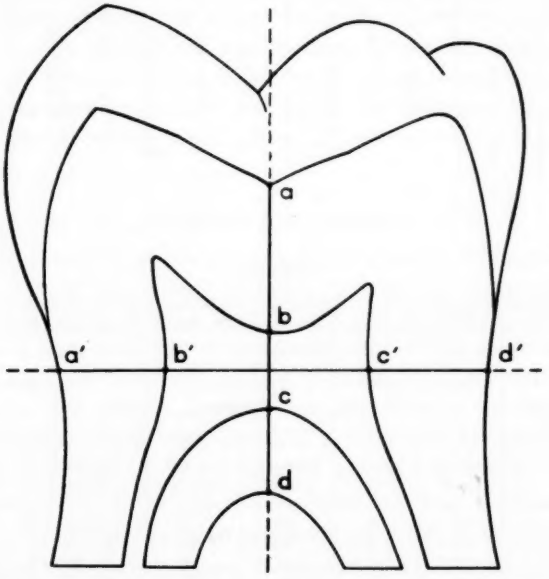

FIG. 2.—Cross-sectional anatomy from the buccal aspect of a left permanent mandibular first molar and technique for obtaining measurements.

Radiographs were taken as follows. In each case the film was placed close to the tooth and its overlying tissues and parallel to its long axis, without bending the film. The distance from target to film was 8 inches, with the central ray perpendicular to the long axis of the tooth from the buccal aspect, passing through the middle of the cemento-enamel junction at the cervix, and parallel with the interproximal spaces of the neighboring teeth. Under these conditions, all radiographs were taken using 65 Kvp at 10 Ma. Each radiograph was then magnified $\times 10$ by means of a projector specially set up in a standard horizontal position, the projection being at right angles to the screen to minimize inaccuracies. Diagrams were drawn on these enlargements for each case. Millimetric measurements were then taken as follows.

Two axes (Fig. 2) were considered: (1) Axis *a–d*, passing through the dentino-enamel junction at the occlusal surface of the tooth and, specifically, through the center *a*, to the center *d* of the gingival extremity at the bifurcation of the roots; and (2) axis *a′–d′*, running perpendicular to *a–d*, in each case bisecting *b–c* (minimum height of the pulp chamber). The *a′–d′* axis approximately coincided with the mesio-distal diameter of the tooth (crown) at the cervix.

The following measurements were taken on these two axes: (1) *a–b*, the thickness of dentin at the roof of the pulp chamber; (2) *b–c*, the height of the pulp chamber; (3) *c–d*, the thickness of dentin at the floor of the pulp chamber; (4) *a–d*, the total distance from the dentino-enamel junction to the bifurcation of the roots; (5) *a′–b′*, the thickness of the mesial (axial) wall of dentin; (6) *b′–c′*, the width of the pulp chamber; (7) *c′–d′*, the thickness of the distal (axial) wall of dentin; and (8) *a′–d′*, the total mesiodistal (width) diameter of the tooth at the cervix. The material was subjected to statistical analysis.

RESULTS

Tables 1 and 2 reveal the differences between the two groups of observations with respect to age and occlusal wear and show why it was necessary to use both of them in our study. Thus the contemporary molars were of known age, whereas for the ancient skeletal molars the ages were not known to a close degree of accuracy,[15, 16] particularly as 77 per cent of the cases fell into the higher-age groups. Moreover, the mean degree of occlusal wear in both groups seemed to increase with age, but not at the same rate (Table 1). Thus the observed contemporary molars displayed a significantly lower degree of occlusal wear than did the ancient skeletal ones, besides which occlusal wear seemed to appear at a later age in the former case than in the latter. More specifically, only 15 per cent of the ancient skeletal molars exhibited occlusal wear less than degree II (Table 2), while the corresponding percentage among the contemporary molars was 56—a difference which becomes still more impressive if we consider that the total age range of the contemporary molars examined was from six to seventy years, whereas for those of the skeletal material it was from five to forty-nine years. Similar conclusions emerged from an examination of the age range corresponding to each degree of occlusal wear (Table 2). This is wider for the contemporary material than for the ancient skeletal observations, chiefly because the upper end of the range probably corresponded to a rather greater age in the former case than in the latter, although again one must bear in mind the limitations of age determination for ancient skeletal material.

In order to illustrate the association between each of the variables of classification and the various tooth measurements under study, Tables 3 and 4 were drawn up.

Vertical measurements.—An examination of Tables 3 and 4 reveals that, regardless of variables of classification, measurement *a–b* does not display a systematic change in either group of observations, except possibly for a trivial upward trend with respect to age observed in the ancient skeletal material. One might contend, however, that even this may be spurious, arising from inaccuracies in the determination of age at death for the ancient anthropological material. Consequently, our measurements permit the formulation of a preliminary hypothesis, according to which (and as far as

TABLE 3

VERTICAL AND HORIZONTAL MEAN MEASUREMENTS (IN MM.) ON FIRST LEFT MANDIBULAR PERMANENT MOLARS OF CONTEMPORARY AND ANCIENT SKELETAL MATERIAL CLASSIFIED BY AGE

Type of Material	No. of Cases	Vertical Measurements					Horizontal Measurements			
		Age Groups (1)	Thickness of Dentin at Roof of Pulp Chamber *a–b* (3)	Height of Pulp Chamber *b–c* (4)	Thickness of Dentin at Floor of Pulp Chamber *c–d* (5)	Over-all Length from Dentino-enamel Junction to Bifurcation of Roots *a–d* (6)	Thickness of Mesial Wall of Dentin *a'–b'* (7)	Width of Pulp Chamber *b'–c'* (8)	Thickness of Distal Wall of Dentin *c'–d'* (9)	Over-all Mesio-distal Diameter of Tooth at Cervix *a'–d'* (10)
Contemporary..	9	6–10	3.13	1.46	1.60	6.19	2.25	4.28	2.43	8.96
	19	11–20	3.12	1.34	2.14	6.60	2.48	3.89	2.50	8.87
	19	21–30	3.19	0.86	2.41	6.46	2.67	3.64	2.66	8.97
	12	31–40	3.19	0.72	2.56	6.47	2.68	3.45	2.72	8.85
	16	41–50	3.11	0.47	2.71	6.29	2.90	3.40	2.76	9.06
	9	51–60	3.40	0.35	2.66	6.41	2.84	3.70	2.68	9.22
	9	61–70	3.37	0.25	3.26	6.88	2.85	3.33	2.70	8.88
Ancient...	9	5–10	3.03	2.18	1.47	6.68	2.18	4.73	2.20	9.11
	8	11–20	3.19	1.15	2.37	6.71	2.60	3.59	2.48	8.67
	27	21–30	3.35	0.67	2.77	6.79	2.71	3.71	2.63	9.05
	20	31–40	3.37	0.40	2.95	6.72	2.79	3.49	2.64	8.95
	11	41–50	3.56	0.48	2.83	6.82	2.85	3.73	2.64	9.22

our material is concerned) neither age nor occlusal wear seemed to affect the thickness of dentin at the roof of the pulp chamber (*a–b*) significantly over the age ranges and degrees of occlusal wear considered.

Contrariwise, the examination of the group mean measurement *b–c* with respect to age, as also with respect to occlusal wear, indicates a change inversely proportional to both variables of classification and of equal intensity for both groups of observations. The same holds true, but conversely, for measurement *c–d;* i.e., the thickness of dentin at the floor of the pulp chamber increased with age and/or occlusal wear. All these changes took place without the over-all measurement *a–d* being affected in any systematic fashion.

Horizontal measurements.—With regard to horizontal measurements, although the

evidence afforded by Tables 3 and 4 is less clear, especially with respect to measurements a'–b' and c'–d', they suggest that the dentin of the mesial and distal walls increased in thickness with age and occlusal wear, while the width of the pulp chamber decreased mesiodistally.

Further, it emerges both from the examination of the group means and from the comparison of the grand means of both groups of observations that the over-all mesiodistal diameter of the tooth at the cervix (a'–d') (Tables 3 and 4) not only displayed a remarkable stability with respect to both variables of classification, as was expected, but also appeared to coincide with the average as determined by other investigators,[10]

TABLE 4

VERTICAL AND HORIZONTAL MEAN MEASUREMENTS (IN MM.) ON FIRST LEFT MANDIBULAR PERMANENT MOLARS OF CONTEMPORARY AND ANCIENT SKELETAL MATERIAL CLASSIFIED BY DEGREE OF OCCLUSAL WEAR

Type of Material	No. of Cases	Vertical Measurements					Horizontal Measurements			
		Degree of Occlusal Wear	Thickness of Dentin at Roof of Pulp Chamber a–b	Height of Pulp Chamber b–c	Thickness of Dentin at Floor of Pulp Chamber c–d	Over-all Length from Dentino-enamel Junction to Bifurcation of Roots a–d	Thickness of Mesial Wall of Dentin a'–b'	Width of Pulp Chamber b'–c'	Thickness of Distal Wall of Dentin c'–d'	Over-all Mesiodistal Diameter at Cervix of Tooth a'–d'
		(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Contemporary..	15	0	3.14	1.33	1.88	6.35	2.36	4.11	2.49	8.96
	37	I	3.24	0.86	2.52	6.62	2.71	3.59	2.66	8.96
	18	II	3.07	0.79	2.49	6.35	2.79	3.53	2.71	9.03
	11	III	3.12	0.61	2.73	6.46	2.64	3.78	2.68	9.10
	8	IV	3.37	0.33	2.71	6.41	2.64	3.24	2.60	8.48
	4	V	3.27	0.30	2.73	6.30	2.89	3.76	2.60	9.25
Ancient...	4	0	3.05	2.67	1.03	6.75	2.05	5.39	1.95	9.39
	7	I	3.26	1.67	2.04	6.97	2.51	4.04	2.39	8.94
	26	II	3.28	0.67	2.75	6.71	2.70	3.72	2.65	9.07
	19	III	3.50	0.67	2.75	6.92	2.73	3.59	2.67	8.99
	15	IV	3.28	0.45	2.82	6.55	2.77	3.54	2.61	8.92
	4	V	3.40	0.40	2.99	6.79	2.78	3.41	2.50	8.69

i.e., at 9 mm. This suggests that the technique used for obtaining measurements from projected radiographs is in fact presenting us with the desired cross-sectional anatomy of the tooth at the cervix, from the buccal aspect.

However, the aforesaid hypotheses regarding changes in the various measurements on the teeth studied were formulated on the basis of observations of the group means, with consequent loss of information; moreover, the observed changes were the result of the combined influences of both variables of classification, which were highly correlated, as a study of Tables 1 and 2 indicates.

We did not, however, test for the significance of the correlation coefficient between the two variables of classification and the measurements made. First, the conditions for the application of "Analysis of Variants" methods are that the observations must

be random and the parent population normal, neither of which condition was obtained in the present case. Second, even if we had employed the large-sample test for the significance of the correlation coefficient, [S.E.r (standard error of r) $\simeq 1/\sqrt{n}$, where n = size of sample] dispensing with the normality assumption above, our test would still have been invalidated by the absence of the first condition, that of randomness of observations.

It was thus felt desirable to verify and estimate with greater accuracy the trends that had been suggested by the examination of the group means of tooth measurements classified with respect to age and occlusal wear and to isolate and estimate for each case the uncontaminated influence of each of the two variables of classification. We therefore proceeded to the computation of the total correlations between every one of the tooth measurements and each of the variables and then the partial correlations, which measure the net uncontaminated influence of each of the variables.

TABLE 5

TOTAL CORRELATIONS BETWEEN AGE, OCCLUSAL WEAR, AND TOOTH MEASUREMENTS

Measurements	Contemporary $r_{12}=+0.6290$		Ancient Skeletal $r_{12}=+0.6074$	
	Age/Tooth Measurements	Wear/Tooth Measurements	Age/Tooth Measurements	Wear/Tooth Measurements
Vertical.....	r_{13}*$=+0.2080$	r_{23}†$=+0.0513$	r_{13}*$=+0.3809$	r_{23}†$=+0.1290$
	$r_{14}=-0.7479$	$r_{24}=-0.5100$	$r_{14}=-0.7152$	$r_{24}=-0.6031$
	$r_{15}=+0.6781$	$r_{25}=+0.3641$	$r_{15}=+0.4727$	$r_{25}=+0.3750$
	$r_{16}=+0.1181$	$r_{26}=-0.0547$	$r_{16}=+0.0866$	$r_{26}=-0.0935$
Horizontal...	$r_{17}=+0.5143$	$r_{27}=+0.2200$	$r_{17}=+0.5389$	$r_{27}=+0.3639$
	$r_{18}=-0.4095$	$r_{28}=-0.2290$	$r_{18}=-0.4305$	$r_{28}=-0.4789$
	$r_{19}=+0.3703$	$r_{29}=+0.1121$	$r_{19}=+0.3974$	$r_{29}=+0.2862$
	$r_{110}=+0.0530$	$r_{210}=-0.0303$	$r_{110}=+0.0324$	$r_{210}=-0.1355$

* $r^2_{13} \simeq 0.04$ (contemporary) and 0.15 (ancient).
† $r^2_{13} \simeq 0$ (contemporary) and 0.02 (ancient).

The symbols adopted range from 1 to 10. Thus (1) signifies age, (2) occlusal wear, (3) thickness of dentin at the roof of the pulp chamber, (4) height of the pulp chamber, (5) thickness of dentin at the floor of the pulp chamber, (6) over-all distance from the dentino-enamel junction to the bifurcation of the roots, (7) thickness of the mesial wall of dentin, (8) width of the pulp chamber, (9) thickness of the distal wall of dentin, and (10) over-all mesiodistal diameter of the tooth at the cervix.

Thus in our text and tables, correlations are denoted (for example) as follows: r_{13} stands for total correlation (Table 6) between age and thickness of dentin at the roof of the pulp chamber. Similarly, $r_{14.2}$ stands for partial correlation (Table 7) between age and height of pulp chamber, with occlusal wear held constant.

The total correlations (Table 5) offer statistical corroboration of the finding that the thickness of dentin at the roof of the pulp chamber (that is, from the fixed center a to the fixed center b) was independent of the degree of occlusal wear, the corresponding r^2_{23}* being practically nil and 0.02 for the contemporary and the skeletal material,

* r^2 being taken as the measure of the proportion of the variability of either of the two variables attributable to the presence of the other.

respectively. On the other hand, the influence of age, although far stronger, was still too small to be of any statistical significance, r^2_{13} being equal to 0.04 and 0.15, respectively.

Again, computations based on individual observations confirmed what the examination of group means had already suggested, revealing a high correlation between the two variables of classification and the height of the pulp chamber. Moreover, the correlations with age were greater than those with wear, r_{14} being —0.75 and —0.72, as against —0.51 and —0.60 for r_{24}, for the contemporary and skeletal material, respectively. The situation with r_{15} and r_{25} was similar, the only difference being that these correlations were positive and weaker. As expected, the r_{26} correlations were very nearly zero.

Regarding the horizontal measurements, the correlations were less pronounced, r_{17} being the strongest, followed by r_{18} and r_{19}. Although the correlations with age were remarkably stable as between contemporary and ancient skeletal material, those with

TABLE 6

PARTIAL CORRELATIONS BETWEEN AGE, OCCLUSAL WEAR, AND TOOTH MEASUREMENTS

Measurements	Contemporary Material		Ancient Skeletal Material	
	Age/Tooth Measurements (Wear Constant)	Wear/Tooth Measurements (Age Constant)	Age/Tooth Measurements (Wear Constant)	Wear/Tooth Measurements (Age Constant)
Vertical.....	$r_{13.2} = +0.2263$ $r_{14.2} = -0.6386$ $r_{15.2} = +0.6202$ $r_{16.2} = +0.1964$	$r_{23.1} = -0.1046$ $r_{24.1} = -0.0767$ $r_{25.1} = -0.1092$ $r_{26.1} = -0.1671$	$r_{13.2} = +0.3840$ $r_{14.2} = -0.5507$ $r_{15.2} = +0.3326$ $r_{16.2} = +0.1813$	$r_{23.1} = -0.1394$ $r_{24.1} = -0.3039$ $r_{25.1} = +0.1256$ $r_{26.1} = -0.1846$
Horizontal.....	$r_{17.2} = +0.4956$ $r_{18.2} = -0.3500$ $r_{19.2} = +0.3881$ $r_{110.2} = +0.0928$	$r_{27.1} = -0.1552$ $r_{28.1} = +0.0413$ $r_{29.1} = -0.1673$ $r_{210.1} = -0.0819$	$r_{17.2} = +0.4297$ $r_{18.2} = -0.2002$ $r_{19.2} = +0.2938$ $r_{110.2} = +0.1458$	$r_{27.1} = +0.0547$ $r_{28.1} = -0.3032$ $r_{29.1} = +0.0615$ $r_{210.1} = -0.1955$

wear showed discrepancies. Here too, however, the correlations with the over-all measurements were practically zero for both age and wear.

The results obtained for the partial correlations (Table 6) seemed to bolster the preliminary conclusions borne out by a scrutiny of Tables 3, 4, and 5. The four partial correlations with respect to the height of the pulp chamber ($r_{14.2}$, $r_{24.1}$), for instance, were always smaller than the corresponding total ones (Table 5). Despite this, the decrease was smaller in the case of age, where the partial correlations still remained high, than in the case of occlusal wear, where they became considerably lower or very nearly vanished (compare r_{14} with $r_{14.2}$ and $r_{24.2}$ with $r_{24.1}$ in Tables 5 and 6). The same held true, but conversely, for measurement *c–d*.

It should be noted that the correlations with age seemed to verify the hypotheses about the independence of measurement *a–b* of the factor of age and the close relationship between age and measurements *b–c* and *c–d* better in the case of contemporary material than in that of the ancient skeletal material. This discrepancy may simply have been due to inaccuracies in estimation of age at death for the latter.

Finally, it was decided to attempt to determine the relative rates of formation of

dentin on the walls considered, over the age range covered by our observations. For this to be possible, however, it was necessary to know the exact ages of the teeth under study, and we were thus compelled to limit ourselves to the contemporary material. We therefore split the contemporary material into two groups, an early group (up to twenty-five years) and a late group (from twenty-five years onward), and observed (Table 7) the behavior of the group correlations as compared with the correlations for the total number of observations, which were used as a standard.

Regarding the vertical measurements, the correlation of age with tooth measurement 3 (a–b) almost disappeared for the early age group but seemed somewhat stronger for the late group. For tooth measurement 4 (b–c), this correlation became similarly attenuated for both age groups, while for tooth measurement 5 (c–d) the correlation showed a dramatic increase for the early age group, becoming much smaller for the late age group.

TABLE 7

TOTAL AND PARTIAL CORRELATIONS BETWEEN AGE AND TOOTH MEASUREMENTS
(Contemporary Material Only)

	Total Correlations			Partial Correlations	
	Early Age Group (Up to 25 Years)	Late Age Group (Over 25 Years)		Early Age Group (Up to 25 Years)	Late Age Group (Over 25 Years)
r_{13}	+0.0534	+0.2200	$r_{13.2}$	+0.1032	+0.2330
r_{14}	−0.0547	−0.5000	$r_{14.2}$	+0.4930	−0.4780
r_{15}	+0.8374	+0.0940	$r_{15.2}$	+0.8053	+0.1097
r_{17}	+0.6010	+0.1731	$r_{17.2}$	+0.5503	+0.2562
r_{18}	−0.3673	−0.0751	$r_{18.2}$	−0.2753	+0.1193
r_{19}	+0.2918	−0.0418	$r_{19.2}$	+0.3246	−0.0048

On the other hand, for the horizontal measurements, the correlation with age for tooth measurement 7 (a'–b') was intensified for the early age group, at the expense of the late age group, which was considerably lower. For tooth measurement 8 (b'–c'), the correlation with age almost disappeared for the late age group, while for the early age group it did not in fact become stronger than the total correlation for the same measurement (see Table 5). Finally, the correlation with age for tooth measurement 9 (c'–d') almost disappeared for the late age group but seemed somewhat stronger for the early group. The corresponding partial correlations exhibited similar behavior.

DISCUSSION

On the basis of this study, it became evident that the formation of dentin was a continuous process throughout the age range studied. This continuous formation was observable in the examined molars of both groups, i.e., those of contemporary individuals, which had been but little exposed to occlusal wear, as well as those from ancient skeletal material, where the teeth had been subjected to the highest degree of occlusal wear considered in this study. The formation of dentin seemed to go on uninterruptedly and on all dentin walls throughout the life of the tooth, taking place faster during the early years of its growth but more slowly later on, the exact rate at any one time depending on the particular dentin wall concerned. Thus (Fig. 3), a five-year-old speci-

men (3, *A*) showed dentin formation up to this age as having taken place at considerable speed, especially on the roof, less on the mesial and distal walls of dentin, with an island of dentin present on the floor of the pulp chamber at the bifurcation of the roots. Specimens in the six to ten-year age range (3, *B*) showed dentin formation occurring at considerable speed on the floor, as well as on the mesial and distal walls of dentin, while that on the roof of the pulp chamber was proceeding at a slower rate. Specimens in the eleven to twenty-year age range, again (3, *C*), showed an over-all decrease in dentin formation rate, the relatively greatest decrease being again at the roof and the least on the floor of the pulp chamber. The progress of these dentin formations continued at the same relative gradients of decrease for the remaining age groups studied. All the walls of the pulp chamber studied showed a continuously declining rate of dentin formation with increasing age. For any one age group, however, the amount of dentin formation was always greatest on the floor and less on the mesial and distal walls. Surprisingly, it was always least on the roof of the pulp chamber itself.

A possible explanation is that, so far as the roof of the pulp chamber is concerned,

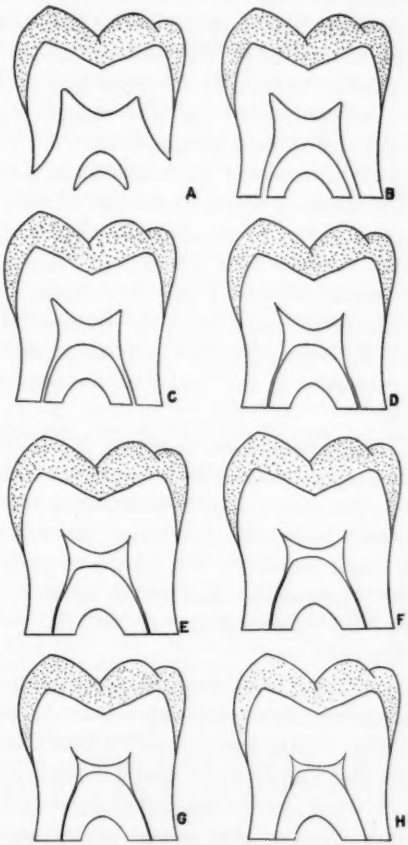

FIG. 3.—Successive stages in growth of a left permanent mandibular first molar, showing dentin formation and reduction in size of pulp chamber with age (*A–H*).

the process of dentin formation had already been virtually completed at an age corresponding to the youngest age groups (see Tables 3 and 4), which was long before the teeth were subject to wear with consequent exposure of dentin. The dentin at this point continued to exhibit no significant quantitative change right up to the latest age groups. This was true regardless of occlusal wear up to degree *V*, where traces of enamel were preserved at the dentino-enamel junction at the center of the occlusal surface of the tooth.

This observation clashes with previously expressed views, according to which, to counteract the influence of occlusal wear, nature causes the formation of a layer of dentin (the so-called "secondary dentin") opposite the abraded area, that is between the injury and the pulp, as if to protect the vitality of the tooth. It has been propounded, moreover, that the pulp of the tooth receded along with advancing occlusal wear, in other words, that the reduction in size of the pulp chamber proceeded chiefly from above, with the formation of a substantial layer of dentin on the roof of the pulp chamber between the injury and the pulp.

Owing to the fact that occlusal wear increased, whereas the pulp chamber became smaller with advancing age, the reduction in size of the pulp chamber has been simply attributed to the coexistent occlusal wear. This fallacy can perhaps be accounted for by the failure of previous workers to examine simultaneously both the varying rates of dentin formation on the respective walls and the relative significance of age and occlusal wear in the changing size of the pulp chamber.

If occlusal wear were the basic cause of the reduction in size of the pulp chamber, there should be a greater formation of dentin at the roof of the pulp chamber, which is not the case. In fact, the thickness of dentin at the roof of the pulp chamber remained almost unaffected by age and occlusal wear, whereas it was the thickness of dentin at the floor which increased considerably with age, over the age range considered, and caused the observed reduction in the size of the pulp chamber occlusogingivally. Meanwhile, the distance between the cemento-enamel junction at the center of the occlusal surface and the gingival extremity at the center of the bifurcation of the roots remained practically the same.

Black observed[11] that "the formation of secondary dentin took place in the same way and to the same extent on the teeth of the same person which were abraded as on those which were not, due to the extraction of their opposing teeth many years before." Our findings are in agreement with this observation, as well as with that of other investigators,[7, 8] although Black attributes this phenomenon to "reflex action." The present data offer a simpler explanation—that of the influence of age, an interpretation that also explains why "the pulp chambers of both the unworn and the abraded teeth were obliterated in the same way."[11]

Through these dentin formations, in the first place, we got a decrease in the size of the pulp chamber of the tooth with increasing age, a decrease which was greater vertically than it was mesiodistally. During this process, it seems that the pulp horns were gradually squeezed between the thin layer of dentin formed on the roof of the pulp chamber and that formed on the upper halves of the mesial and distal walls of dentin. The root canals were further squeezed with advancing age, as a result of the dentin formed on the lower halves of the mesial and distal walls of dentin, together with the

greater mass of dentin at the floor of the pulp chamber. This resulted in their narrowing down and even, on occasion, in their obliteration.

SUMMARY

Dentin formation and size of the pulp chamber were studied in 168 sound, left, permanent mandibular first molars of both sexes, belonging to contemporary individuals, and ancient skeletal material of different age groups suffering varying degrees of occlusal wear. These teeth were examined clinically and radiographed so as to show their cross-sectional anatomy from the buccal aspect under identical standard conditions. The resultant radiographs were enlarged ten times, and diagrams were drawn. Measurements were then taken by means of eight defined indexes and analyzed. The data were tabulated separately for the contemporary and ancient skeletal observations, according to age groups and assessed degrees of occlusal wear. Dentin formation and the size of the pulp chamber under the influence of the two factors—age and occlusal wear—were investigated. The material was subjected to statistical analysis.

The main findings were as follows: (1) There appeared to be no significant change in the dentin at the roof of the pulp chamber with increasing age over the entire spans of age and occlusal wear studied. (2) The dentin at the floor of the pulp chamber showed a definite increase in thickness with increasing age, this being chiefly responsible for the reduction in size of the pulp chamber occlusogingivally. (3) The mesial and distal walls of dentin increased in thickness with increasing age, causing the reduction in size of the pulp chamber mesiodistally. (4) Both the height and the width of the pulp chamber showed a definite decrease with advancing age, regardless of the degree of occlusal wear studied.

It thus appears that, regardless of occlusal wear up to degree *V*, dentin formation goes on uninterruptedly throughout the life of the sound tooth, faster in the early years but more slowly later on. It is this continuous formation of dentin with age that is responsible for the decrease in size of the pulp chamber.

The author is indebted to the curators of the following museums for facilities in obtaining and examining the ancient skeletal material referred to in the text: (1) Museum of the Ancient Agora of Athens, (2) Anthropological Museum of the National University of Athens, (3) Archaeological Museum of Nauplion, and (4) Museum of the Keramikos, Athens. The author wishes to thank Mr. P. Pappas for the statistical analysis of the data.

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Reactions of Tin (II) and Fluoride Ions with Etched Enamel

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For many years the occurrence and distribution of fluoride in human teeth have been compelling subjects of dental research. Realization of the benefits of fluoridated water supplies gave new impetus to the study of fluoride in teeth. More recently there has been much attention focused on topical treatment of teeth with solutions or pastes containing fluoride as sodium fluoride or tin (II) fluoride. Topical fluoride treatments have considerable biologic and practical dental significance. In addition, the reactions occurring between the treating agents and the enamel raise chemical questions of fundamental importance. This article describes studies of the rates of the topical reactions on uniform, etched enamel surfaces.

The distribution of fluoride in human enamel has been examined and discussed by Brudevold, Gardner, and Smith.[1] These authors, in agreement with others, pointed out that enrichment of fluoride in a thin outer layer of enamel is a general phenomenon. The concentration of fluoride at the very surface seems to approach a limit of about 1 per cent of the enamel by weight, a value that may be the "physiological saturation" point.

Nearly all the work on the reaction of fluoride with enamel has dealt with the effects of treating powdered enamel with sodium fluoride solutions. A thorough investigation of this subject has been made by Leach,[2] who used sodium fluoride solutions of widely varying concentrations. Both powdered enamel and powdered dentin react with very dilute fluoride solutions to yield fluorapatite, in which the fluoride ion occupies the lattice position held by hydroxide ion in hydroxylapatite, the principal component of the powdered enamel and dentin:

$$\underset{\text{hydroxylapatite}}{Ca_5(PO_4)_3OH} + \underset{\text{(dilute)}}{F^-} \rightarrow \underset{\text{fluorapatite}}{Ca_5(PO_4)_3F} + OH^-$$

At higher concentrations of fluoride, the main reaction is one of metathesis, with calcium fluoride as a product:

$$\underset{\text{(more concentrated)}}{Ca_5(PO_4)_3OH} + 10F^- + 3H^+ \rightarrow 5CaF_2 + 3HPO_4^= + OH^-$$

The over-all reactions, of course, are much more complicated than one would infer from these simplified equations. The principle of formation of fluorapatite at low fluoride concentrations and calcium fluoride at higher concentrations has often been stated by others. McCann and Bullock[3] have also mentioned the possibility that fluorapatite may be a minor product at high fluoride levels and that fluoride could also

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react by direct adsorption. Since magnesium and carbonate are minor ingredients of enamel, some fluoride could also react by exchange with carbonate or precipitation as magnesium fluoride.

Reactions between fluoride ion and whole teeth or enamel chips have been demonstrated by several research groups.[4–8] Hein, Bonner, Brudevold, and Hodge[9] reported that freshly extracted teeth took up fluoride from very dilute solutions (F^-, 1 p.p.m.) more rapidly during the first few minutes of exposure than later. After the surfaces were pumiced, they took up somewhat more fluoride, and after several pumicings the amount taken up in a 5-minute treatment rose sharply. According to Norvold, Inglis, and Armstrong,[10] immersion of teeth in sodium fluoride solution containing 0.1 per cent fluoride results in more fluoride taken up by exposure for 200 minutes than for 100 minutes. Teeth in the mouth have been shown by Adler, Straub, and Popovics[11] to take up measurable amounts of fluoride from 0.001–0.002 per cent sodium fluoride solutions in 15–20 minutes.

The reaction of tin (II) ions with powdered or intact enamel has been considered only recently. Brudevold and Steadman[12] found that erupted human teeth contained a very small amount of tin (7 p.p.m. or less) in the outermost layer and virtually none in deeper layers. Several reports have furnished qualitative evidence for the formation of a surface coat on enamel treated with tin (II) fluoride solution. Recently Scott[13] has used electron microscopy and electron diffraction to detect such a coat and to identify calcium fluoride and a tin compound in it. Nebergall and Langer[14] confirm the presence of calcium fluoride when tin (II) fluoride is allowed to react with powdered or whole enamel. These investigators show that the other product is a tin (II) hydroxy-orthophosphate.

The work of Brudevold, Steadman, Gardner, Rowley, and Little[15] is especially pertinent to our studies of reaction rates. Brudevold has also referred to this work in a review article.[16] Permanent molars, cleaned by pumicing, were gently shaken with 100 ml. of treating solution for 20 minutes. The solutions, all equivalent with respect to original fluoride concentration, were 1 per cent sodium fluoride, 1.86 per cent tin (II) fluoride, and 4.12 per cent tin (II) chlorofluoride. Layers ground by hand from the treated teeth were analyzed for tin (II) and fluoride. Each layer was probably about 0.005 cm. thick. In the sodium fluoride experiment most of the fluoride was found in the first layer of enamel, although some penetrated to the second layer. Somewhat less fluoride was found in the first layer when either tin (II) fluoride or tin (II) chlorofluoride was the treating agent. About the same amount of fluoride was found in the second layer regardless of which treating agent was used. After treatment with the tin compounds the enamel was found to contain appreciable quantities of tin (II) in both first and second layers, but more in the first than in the second. These findings are compared in detail with our own results in a later section.

EXPERIMENTAL METHODS

All our work has involved the bulk enamel, below the very thin, fluoride-rich outer layer. The pieces of central incisors used in the experiments were consumed almost to the dentin during the course of the work. Any particular experiment, therefore, may have been performed on any part of the enamel with respect to depth, excluding only the outermost layer. The teeth used were all collected from dentists and hospitals in

Cincinnati, then stored in 5 per cent formaldehyde solution made slightly basic with sodium hydroxide. Weighed samples of the experimental enamel ranging from 5,000 to 50,000 μg, contained in no instance more than 1 μg. of fluoride. It will be obvious in later discussion that natural fluoride in the enamel used was negligible. There was also no measurable amount of tin (II) in the enamel.

Square pieces of sound incisor enamel, 0.4 cm. on a side, were cut from the experimental teeth and mounted on the ends of glass rods with dental plastic.* The rods and especially the mounting plastic had to be perfectly smooth to avoid misleading adsorption of treating ions. During each treatment two or three rods were set off-center in a large rubber stopper fastened at its center to the shaft of a stirring motor. The samples were revolved face down in the treating solution.

Before the treatment each sample of enamel was etched for 1 minute in 2 M perchloric acid, since preliminary experiments showed that etched enamel surfaces gave more precise results than did natural or polished surfaces. The treating solutions of sodium fluoride† and tin (II) fluoride‡ were freshly made at room temperature for each test. The various concentrations, pH's, and times of treatment are given with the results. All operations with solutions containing tin (II) were done in a nitrogen-filled box, to minimize oxidation of the tin (II) ions. In early experiments the amounts of tin (II) and fluoride taken up varied erratically because of the accumulation of precipitates and debris on the enamel surfaces. This troublesome variation was overcome by fastening the heads of three toothbrushes, bristles up, to the bottom of the treatment beaker, so that the samples were brushed continuously with the solution as they revolved.

After treatment, an enamel sample for analysis was collected by dissolving layers of the pieces in 2 M perchloric acid. Immersion for 1 minute, with gentle agitation by hand, dissolved 0.01 cm. (100 μ) of the enamel's thickness.§

Fluoride in the acid solution was determined by a method similar to that of Smith and Gardner.[17] The method can detect 1 μg. of fluoride and is precise within 1 μg. Tin (II) was determined by direct iodimetric titration to the electrometric end point. The pH meter|| was fitted with a platinum and a calomel electrode and read on the millivolt scale. This method is so sensitive that 10^{-5} M iodine may be used in the titration; as little as 1 μg. of tin (II) can be detected. The precision limits of the readings are about ± 0.5 μg.

RESULTS AND DISCUSSION

Let us consider, first, the reaction of fluoride with enamel, then the reaction of tin with enamel.

Reaction of fluoride.—Figure 1 presents the results of brushing treatment with 0.1 M tin (II) fluoride and 0.2 M sodium fluoride solutions. The quantities of fluoride shown are corrected for amounts taken up by the tooth mountings, these amounts

* DURA BASE,® Reliance Company, Chicago, Ill.

† Baker's Reagent grade.

‡ Ozark-Mahoning Co.

§ In eight series of etchings on different samples of enamel, the thicknesses of enamel removed in 1.00 minute were 93, 112, 112, 96, 90, 105, 90, 90, and 111 μ. The mean value is 99.9 μ.

|| Beckman Model G.

being less than 1 μg. when the mountings were properly smoothed. Replicate points are shown for some of the determinations, to give an idea of the experimental variation. In general, where only single points are shown, they represent the mean of two or three determinations. The amount of fluoride found in each analysis is reported as the total for as many 0.01-cm. layers as necessary to recover all the fluoride. In the longer treatments some fluoride penetrated to the second and third layers. For this reason it is not very informative to quote the fluoride contents in parts per million. If all the fluoride had remained uniformly distributed in the first layer, 1 μg. of fluoride per square centimeter of surface would correspond to 33 p.p.m.

It is easily seen that the fluoride from either source reacted fairly rapidly during the first few minutes of brushing, then reacted steadily and more slowly thereafter. At the particular concentrations chosen, sodium fluoride supplied more fluoride to enamel

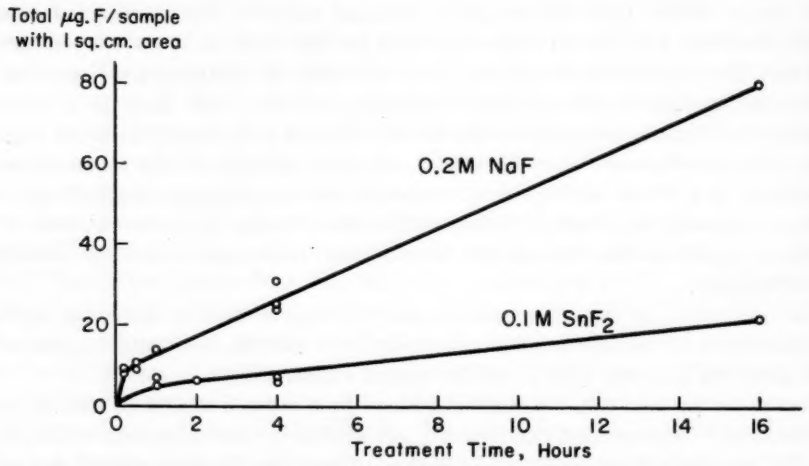

FIG. 1.—Fluoride deposited on intact enamel. Samples were treated by brushing at room temperature in unbuffered solution (pH 2.8 for SnF_2, pH 6–7 for NaF).

after any given treatment time than did tin (II) fluoride. A possible explanation of the difference is suggested below in the discussion of the reaction with tin (II) ion.

Penetration of fluoride into the enamel is described in Figure 2. Here the total amounts of fluoride were subdivided into the amounts for each 0.01-cm. layer. Two important things are brought out by these curves. First, the greater deposits of fluoride after long sodium fluoride treatments mean both that more fluoride had penetrated and that more had remained in the first layer. The longer treatments with tin (II) fluoride laid down most of the added fluoride near the surface. Second, the actual extent of penetration was rather great. For the 16-hour sodium fluoride treatment, 8 μg. of fluoride per square centimeter was found in the third layer, and the bottom of this layer was about 30 per cent of the way through the enamel of the incisor samples.

More evidence that the fluoride from treatment with sodium fluoride was not mostly on the surface of the enamel was provided by brushing tests with an abrasive. After 4 hours of brushing treatment with 0.2 M sodium fluoride solution, samples had taken up about 25 μg. of fluoride per square centimeter. Then the enamel was brushed fur-

ther, but a 50 per cent slurry of $CaHPO_4 \cdot 2H_2O$ was substituted for the sodium fluoride solution. Brushing with this abrasive for any period between 30 minutes and 2 hours removed about one-eighth of the fluoride. Even 16 hours of abrasive brushing removed only about one-fourth of the fluoride. These results are approximate because of the need for a separate treatment for each part of the experiment. Each analysis, of course, removed all the fluoride and several layers of enamel, making direct comparison of brushing times impossible for a single surface.

In the work of Brudevold *et al.*,[15] after whole teeth had been treated with 1 per cent (0.25 M) sodium fluoride, the first enamel layer contained 0.144 per cent fluoride, and

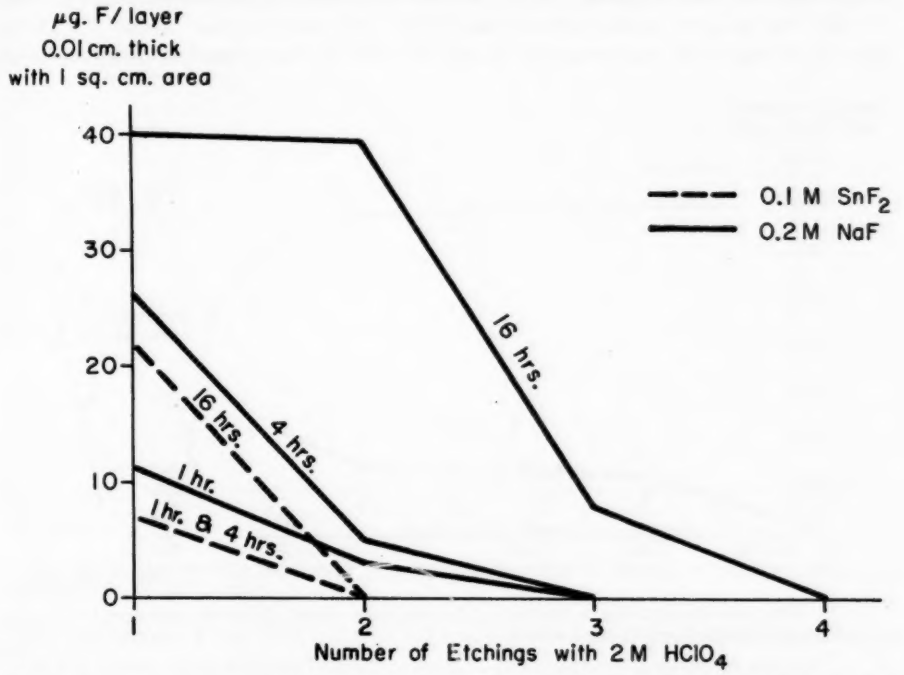

FIG. 2.—Penetration of fluoride into enamel. Each etching represents a layer of enamel 0.01 cm. thick

the second contained 0.0561 per cent. Untreated teeth used as controls contained 0.0924 per cent fluoride in the first layer and 0.0400 per cent in the second. The tin (II) found in the first layer after tin (II) fluoride treatment was 0.0600 per cent; from tin (II) chlorofluoride it was 0.0640 per cent. In the second layer the values were 0.0250 per cent (SnF_2) and 0.0300 per cent (SnClF). No measurable amount of tin was found in untreated teeth. The fluoride in the first layer after tin (II) fluoride treatment was 0.120 per cent, and after tin (II) chlorofluoride treatment, 0.103 per cent. The fluoride in the second layer was 0.0580 per cent (SnF_2) and 0.0570 per cent (SnClF).

Brudevold's findings for treatment with 0.25 M sodium fluoride solutions are very close to ours for brushing with 0.2 M solutions, assuming that each layer of enamel in Brudevold's work was 0.005 cm. thick. The agreement appears when Brudevold's

rather large natural fluoride contents are subtracted from the gross fluoride found after treatment. Brudevold's figures for fluoride taken up from tin (II) fluoride solutions are about twice ours for comparable conditions. Good agreement should not be expected, because Brudevold's shaking treatments and our brushing treatments are most noticeably different when tin (II) is present in the solutions.

The fluoride taken up by teeth in the mouth, as reported by Adler *et al.*,[11] is in the same range as our figures for enamel squares, but the fluoride solutions used in the mouth were very dilute: 0.0002–0.0005 M. At such low concentrations of sodium fluoride in our system we found smaller amounts of fluoride laid down, the amounts increasing with concentration (Fig. 3, *A*). In the middle concentration range (0.005–0.2 M), the reaction rate is almost independent of concentration. Above 0.2 M the rate again rises with concentration. It may be that the rising portion of the curve at

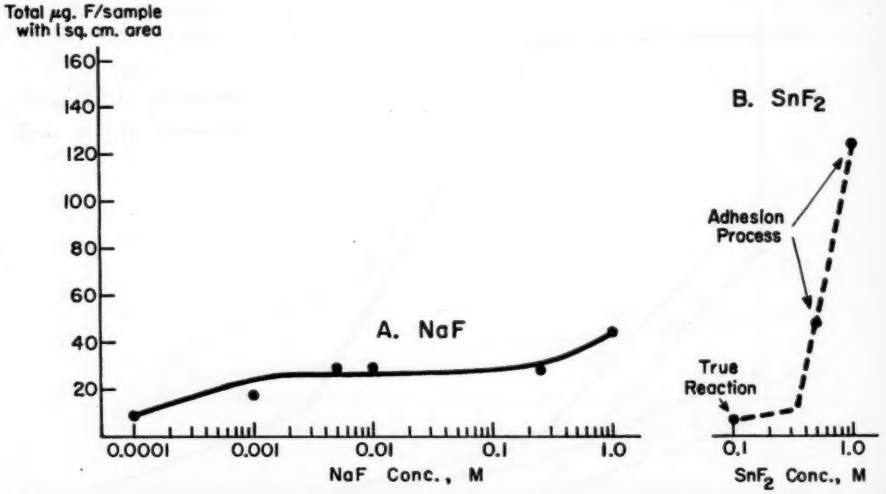

FIG. 3.—Effect of concentration on reaction of fluoride with enamel. Brushing treatment for 4 hours at room temperature in unbuffered solutions.

low concentrations reflects the reaction between fluoride and enamel to produce fluorapatite, while the rising portion at high concentrations corresponds to the formation of calcium fluoride as the main insoluble product. If both reactions contribute to the total, the curve of Figure 3, *A*, represents a composite reaction rate.

Dilute solutions of tin (II) fluoride (below 0.1 M) supplied very small amounts of fluoride to the enamel in 4 hours (Fig. 3, *B*), and no reliable curve could be drawn for such concentrations. Above 0.1 M the rate increased sharply with concentration. These results with stronger solutions were influenced by hydrolysis of the tin ions and formation of precipitate during the treatment, despite the brushing action. Much of the large quantity of fluoride found on the enamel was occluded in the precipitate that adheres to the surface instead of being taken up by direct reaction. Further reference is made to this adhesion process later in the discussion.

Reaction of tin (II).—Figure 4 presents a rate curve for the reaction between etched enamel and 0.1 M tin (II) fluoride solution at natural pH (2.8) and room tempera-

ture. The enamel was continuously brushed with the solution. Quantities of tin (II) were corrected for adsorption by the tooth mountings. Initially, the reaction was fairly rapid, but after a short time a limiting value was reached. Further brushing did not cause any net gain in the amount of tin taken up. The limiting value was not merely a consequence of accumulation of loose film and debris on the surface, blocking further reaction. Accumulation can occur with immersion or stirring treatment, but it was minimized by brushing. Apparently, the limiting value corresponded to the formation of a more tenacious coating, a tin-containing layer that worked its way into the depressions in the enamel and covered the whole surface. This tough layer prevented any further penetration of tin and also slowed the rate at which fluoride could diffuse

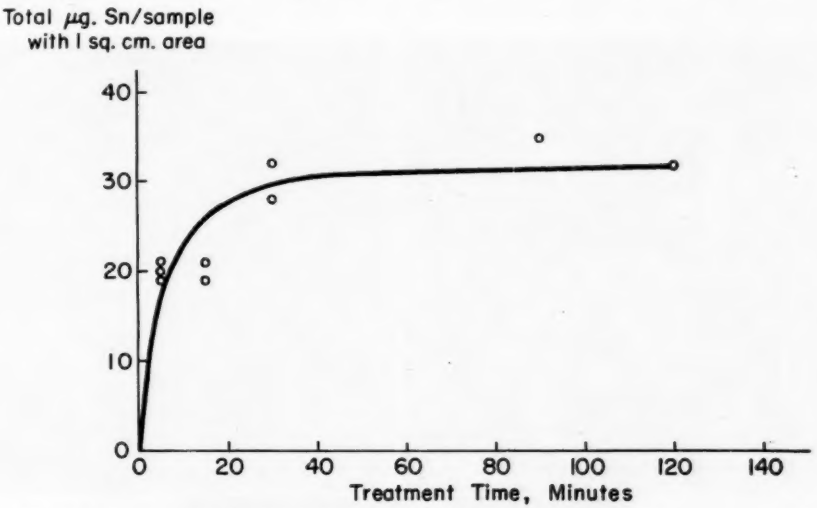

FIG. 4.—Tin deposited on intact enamel. Samples were treated by brushing at room temperature in unbuffered 0.1 M SnF_2 solution.

into the enamel. Thus tin (II) fluoride solutions in which hydrolysis is not extensive are slower than sodium fluoride solutions in supplying fluoride to enamel.

Although the tough coating laid down by the reaction was not visible to the unaided eye, evidence for its existence was given by electron micrographs of platinum replicas of the enamel (Fig. 5). Brushing the etched enamel with water brought out the rod structure, but brushing with tin (II) fluoride solution applied the tin-containing coat and obscured the structural features.

Studies of penetration (Fig. 6) confirmed the idea of a tin deposit confined mostly to the surface. No tin at all was found in the second layer after a 30-minute brushing treatment. Continuing to a 4-hour treatment added a small amount of tin to the first layer and permitted some tin to diffuse into the second layer. The continuous kneading action of the bristles may have facilitated this very slow penetration. After 4 hours the only significant change was copious precipitation of hydrous tin (II) oxide ("stannous hydroxide") onto the surface of the enamel and all other surfaces exposed to the solution—mounting, beaker, and bristles. Long treatment time was one source of hydrolytic precipitation of tin (II) fluoride solutions, and most of the very large

amount of tin recovered for analysis after extended treatment actually consisted of adhering precipitate. We distinguish between the true solution-solid reaction, by which the tenacious coating was laid down on the enamel, and the adhesion reaction, by which precipitate rather loosely coated all surfaces, including enamel already bearing the tough coating.

The tin-containing coat was strongly held by enamel, but its external nature compared with deposited fluoride was evident from abrasive brushing tests. Of the tin (II) laid down by 30-minute brushing, about half could be removed by 15 minutes of brushing with 50 per cent $CaHPO_4 \cdot 2H_2O$. Six hours of brushing removed about three-fourths of the tin. Treatment for 4 hours attached the tin more firmly, so that 3 hours of brushing with abrasive removed slightly less than the 1.5-hour abrasive brushing

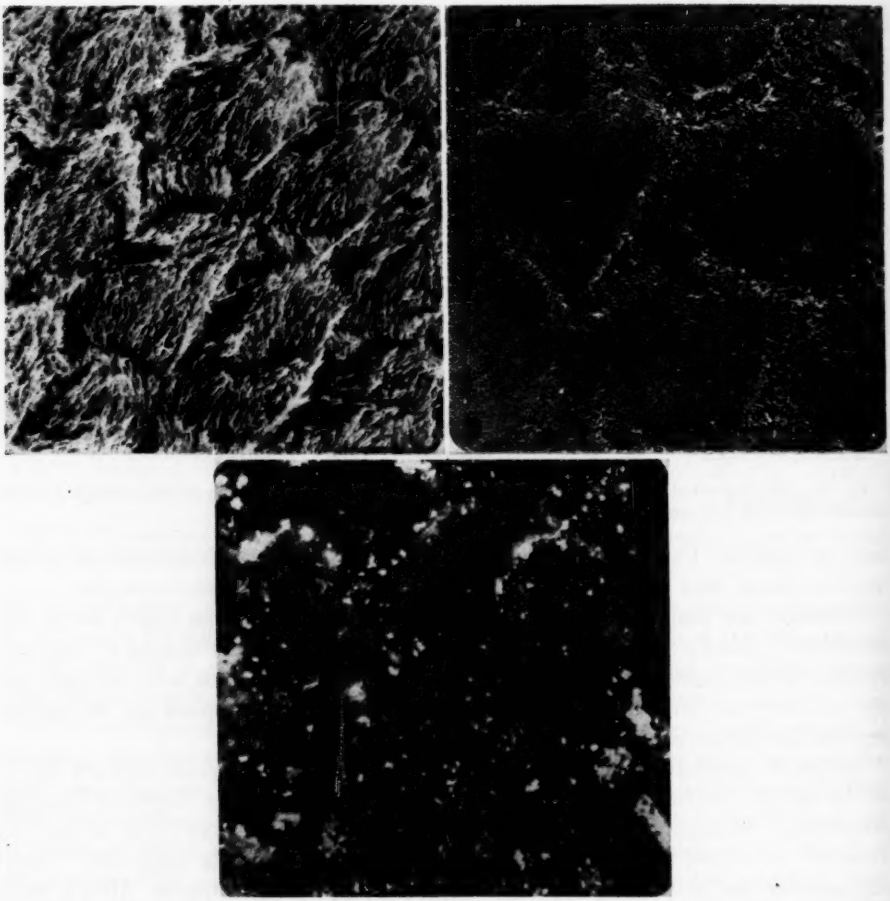

FIG. 5.—Electron micrographs of enamel. All specimens were polished before treatment. *A:* treated with 2 M $HClO_4$ for 1 minute; *B:* treated with 2 M $HClO_4$ for 1 minute, then brushed with water for 30 minutes; *C:* treated with 2 M $HClO_4$ for 1 minute, then brushed with SnF_2 for 30 minutes. (Mag. ×6,000.)

removed after a 30-minute treatment. Severe as these abrasive brushings were, compared with ordinary home toothbrushing, they would not remove much of the fluoride from sodium fluoride treatment, as discussed earlier. The fluoride from tin (II) fluoride treatment, of course, penetrated to about the same extent as the tin, and it presumably would be removed in much the same proportions as was the tin during severe abrasion.

When the total amounts of tin (II) taken up by whole teeth in the experiments of Brudevold *et al.*[15] are compared with our results, the agreement is quite good, even though the conditions of treatment in the two sets of experiments were quite different.

The effect of concentration of the tin (II) fluoride on the reaction rate was studied

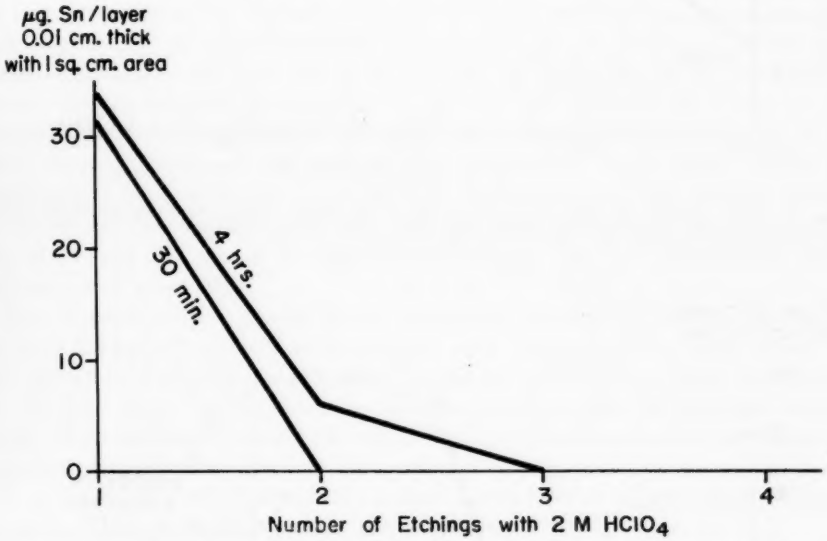

FIG. 6.—Tin content of successive layers of enamel. Brushing treatment with 0.1 M SnF_2. Each etching represents a layer of enamel 0.01 cm. thick.

in another series of tests (Fig. 7). Each solution was used at natural pH. The range of pH was 2.8–3.6 for the concentration range 0.00001–0.1 M, with pH decreasing as concentration increased. The results of 30-minute brushings are given as total tin (II) recovered per square centimeter of enamel surface. Dissolving to a depth of 0.01 cm. was sufficient to recover all the tin (II) in these experiments.

Very low concentrations of tin (II) were sufficient to deposit appreciable amounts on the enamel. The 0.00001 M solution yielded about one-fifth as much as did the four most concentrated solutions. Over the concentration range 0.001–0.1 M the reactivity of tin (II) was nearly constant. We concluded that the 0.001 M solution contained enough tin (II) to lay down a uniform surface coat within 30 minutes. The stronger solutions also produced the coat quickly—perhaps more quickly—but, once it was formed, it blocked further reaction.

For a set of experiments involving changes in pH, the samples of enamel were brushed for 30 minutes with 0.1 M tin (II) fluoride solution (Fig. 8). Adjustments of

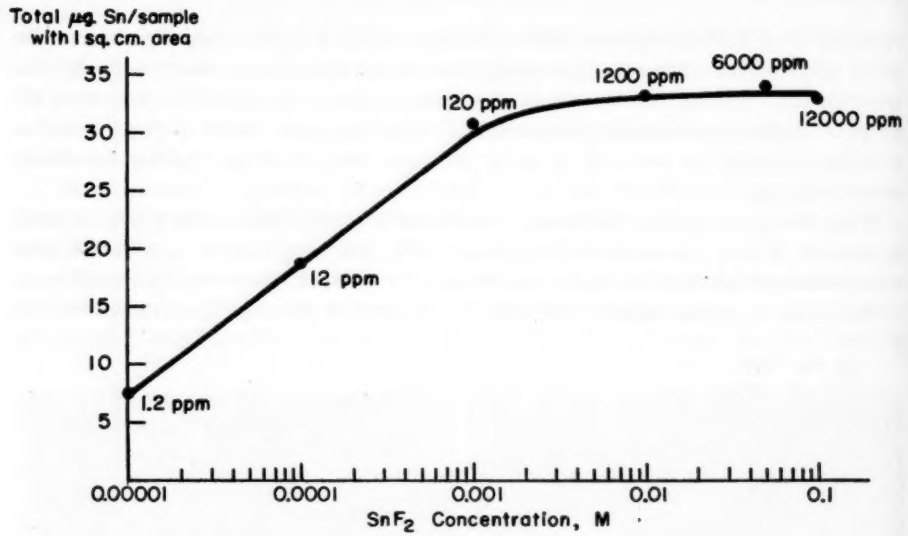

FIG. 7.—Effect of SnF_2 concentration on reaction of tin with enamel. Brushing treatment for 30 minutes at room temperature in unbuffered solutions.

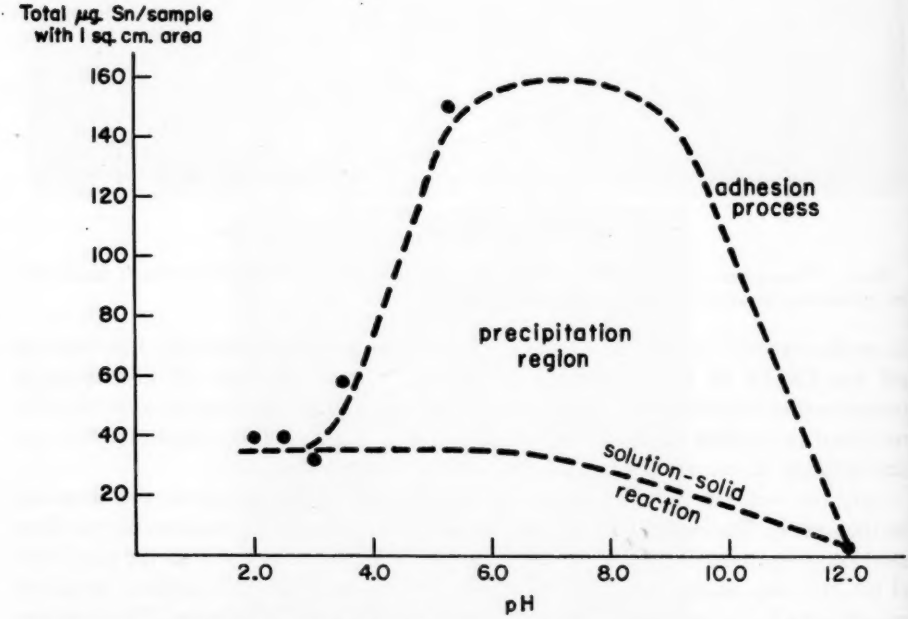

FIG. 8.—Effect of pH on reaction of tin with enamel. Brushing treatment for 30 minutes at room temperature in 0.1 M SnF_2 adjusted with $HClO_4$ or $CO_3^{=}$-free NaOH.

pH were made with perchloric acid or sodium hydroxide. At pH 3 or below, the reactivity of tin (II) changed very little with pH. Solutions below pH 2 were not used, because they attack enamel severely during the treatment. Just above pH 3, the simple aqueous solutions of tin (II) fluoride at 0.1 M hydrolyzed rapidly, precipitating hydrous tin (II) oxide. With partly non-aqueous systems, including toothpastes, the pH threshold is much higher and the rate of precipitation much lower. For example, a solution of 0.4 gm. tin (II) fluoride in 30 gm. of water, 15 gm. of glycerin, and 15 gm. of sorbitol, originally adjusted to pH 4.5, contained 85 per cent of the original tin (II) ions still in solution after 125 days of aging. A solution of 0.4 gm. tin (II) fluoride in 30 gm. of water, originally adjusted to pH 4.5, contained 47 per cent of the tin (II) after 106 days.[18] With the simple solutions of our experiments, the precipitate could be seen gathering on the enamel above pH 3. As the pH was raised further, copious and irregular precipitation covered the enamel with large amounts of tin, obscuring the direct solution-solid reaction. The boundaries of the "precipitation region" could not be well defined. Indirect evidence suggests that the true solution-solid process yielded less and less tin as the pH rose. At pH 12, all the precipitate dissolved, and the tin was in the form of a hydroxide-tin (II) complex ("stannite"). This complex reacted only slightly in 30 minutes. Since it is known that hydroxide complexing becomes stronger as the pH rises, even on the acidic side, and that this complexing lowers the concentration of free tin (II) ions, it is reasonable to conclude that the solution-solid reaction decreases with rising pH.

The adhesion process was distinctive and easily recognized. Precipitated tin (II) oxide on the enamel appeared to the eye as a gray film, whereas the surface coat from the solution-solid process could be detected only by electron microscopy. Surfaces that did not pick up tin by the solution-solid process—glass rods, for example—acquired layers of precipitate. These layers, when fresh, could be dissolved in perchloric acid and titrated with iodine. The treating solution itself unmistakably showed the hydrolysis by its opaque, milky appearance instead of the clear or slightly cloudy aspect of fresh tin (II) fluoride solutions at natural pH.

Any conditions that promote hydrolysis lead to the accumulation of tin (II) by adhesion. Treatment with 0.1 M tin (II) fluoride for periods up to 4 hours did not add much tin (II) to that laid down in 30 minutes. Very long treatment times, allowing hydrolysis to become important, resulted in easily visible precipitate on the enamel and large amounts of tin (II) by analysis. Elevated pH's or highly concentrated solutions had the same kind of effect, but change in pH had a much more drastic influence on rate than had increase in tin (II) concentration. More readily hydrolyzing tin (II) salts, such as tin (II) chloride, reacted partly by the adhesion process, even at their low natural pH's. Treatment by immersion instead of brushing sometimes produced adhesion of precipitate.

SUMMARY

Acid-etched but otherwise sound and uniform samples of human incisor enamel were treated by brushing with sodium fluoride or tin (II) fluoride solutions for various times at different concentrations and pH's. After treatment, the samples were exposed to strong acid to dissolve specific thicknesses of enamel. The acid solutions were ana-

lyzed chemically for tin (II) or fluoride ions. The experiments support the following conclusions.

Both tin (II) and fluoride reacted with enamel much more rapidly during the first few minutes of brushing than they did afterward. Fluoride taken up by enamel from either sodium fluoride or tin (II) fluoride solution was found at appreciable depths in the enamel. Most of the tin (II) from tin (II) fluoride solution was laid down as a uniform coat on the surface or slightly within the enamel.

Tin (II) and fluoride solutions in the concentration range 0.00001–0.001 M deposited measurable amounts of tin (II) and fluoride onto enamel, and the reactivity increased with increasing concentration. Tin (II) or fluoride solutions from 0.001 to 0.2 M showed little change in reactivity with enamel as concentration was varied. As sodium fluoride concentration was raised from 0.2 M to saturation, the reactivity of the fluoride with enamel increased with concentration, reaching a maximum value of 40–50 μg. of fluoride laid down per square centimeter of enamel in 4 hours under the conditions used in this work.

In the reaction of tin (II) with enamel, high tin (II) concentration, high pH, long treatment times, or the presence of rapidly hydrolyzing tin (II) salts favored an adhesion process whereby precipitated tin (II) oxide accumulated on the surface. If fluoride was present, some of it was occluded in the precipitate.

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Delayed Effects of Multiple Sublethal Doses of Total Body X-Irradiation on the Periodontium and Teeth of Mice

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In the course of an investigation of the delayed effects of multiple sublethal doses of total body X-irradiation in the mouse, it was observed that animals which survived a total dose of 1,200 r subsequently developed severe periodontal lesions which were not noted in non-irradiated controls.[1] Qualitatively similar findings have also recently been reported for the rat.[2] The purpose of the present study is to confirm and extend these preliminary findings and to present a histopathologic description of the lesions obtained.

MATERIALS AND METHODS

Three hundred and twenty male mice of the Webster strain were selected in the body-weight range of 11–14 gm. and were provided a purified diet consisting of cerelose, 59 per cent; casein,* 24 per cent; cottonseed oil, 10 per cent; salt mixture,† 5 per cent; and cellulose,‡ 2 per cent. Vitamins were added in the following amounts per kilogram of diet: thiamine hydrochloride, 10 mg.; riboflavin, 10 mg.; pyridoxine hydrochloride, 10 mg.; calcium pantothenate, 60 mg.; nicotinic acid, 100 mg.; ascorbic acid, 200 mg.; biotin, 4 mg.; folic acid, 10 mg.; para-aminobenzoic acid, 400 mg.; inositol, 800 mg.; vitamin B_{12}, 150 μg.; 2-methyl-1,4-naphthoquinone, 5 mg.; choline chloride, 2 gm.; vitamin A, 5,000 U.S.P. units; vitamin D_2, 500 U.S.P. units; and alpha-tocopherol acetate, 100 mg. The vitamins were substituted for an equal weight of cerelose in the diet.

The animals were placed in metal cages with raised screen bottoms (5 animals per group) and were provided with food and water ad libitum. The mice were fed daily, and any food not consumed 24 hours after feeding was discarded. After 6 weeks of feeding, 40 of the mice were selected at random to serve as non-irradiated controls. The remainder received an exposure of 200 r total body X-irradiation which was repeated once weekly until a total dose of 1,200 r (6 exposures) had been administered. The animals to be irradiated were placed in a wooden box divided into 60 equal com-

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* Vitamin-free Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

† Wesson Modification of Osborne-Mendel Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Solka Floc, Brown and Co., Boston, Mass.

partments, 1¼ inches wide, 3 inches long, and 1½ inches deep. The partitions and top were made of ⅛-inch cellulose acetate sheeting. The top and bottom of each compartment were perforated with holes for purposes of ventilation. The container was rotated slowly on an electrically driven turntable to insure equivalent exposures.

The following radiation factors were employed: GE Model Maximar 250; 250 kv; 15 ma; 0.5-mm. Cu and 1-mm. Al filters plus a Cu parabolic filter;* HVL, 2.15-mm. Cu; target distance to top of box, 82 cm.; and dose rate, 15.6 r/min (measured in air, at top of box).†

Representative animals from both the irradiated and the non-irradiated groups were sacrificed 80, 100, 120, 150, 180, and 300 days after the first X-ray exposure. The heads of these animals, as well as the heads of those that died during the course of the experiment, were fixed in Bouin's solution. Sections were prepared both of the head *in toto* and of individual molar quadrants. Whole heads were decalcified in 10 per cent nitric acid in 10 per cent formalin and were washed with saturated lithium carbonate solution. They were then dehydrated and infiltrated in the routine manner, imbedded in 50 per cent nitrocellulose, sectioned at 20 μ thickness, and stained with hematoxylin-eosin or Mallory's triple stain.

In the case of individual molar quadrants, each was freed from the rest of the head prior to demineralization and was examined under a binocular dissecting microscope for gross evidence of pathology. Following this subjective evaluation, examples of molar quadrants representing the entire spectrum of periodontal involvement, as well as those from control animals, were selected for microscopic study. These tissues were demineralized in a 37 per cent aqueous solution of di-sodium EDTA‡ (adjusted to pH 7.4 with HCl) and, after imbedding in nitrocellulose and paraffin, were sectioned at 6 μ. Such sections were stained with hematoxylin and eosin or by the periodic acid–Schiff technique.

In addition to the experiments outlined above, tests were also conducted with 60 irradiated and 20 non-irradiated mice fed a natural food stock ration§ but otherwise treated identically with the above. Surviving animals in this series were sacrificed 120 days after the first X-ray exposure.

RESULTS

Body weight and survival.—Sixty-eight per cent of the irradiated mice on the purified diet and 80 per cent of those on the stock ration succumbed within 100 days of the first X-ray exposure. Average survival times of the decedents in these groups were 45.5 and 39.4 days, respectively. By contrast, less than 10 per cent of the non-irradiated mice on each of the diets tested succumbed during a comparable period. With

* A non-uniform filter which produces a flat isodose surface of X-ray intensity constructed by the method of Greenfield and Hand (see Ref. 3). We are indebted to Dr. Moses Greenfield and Miss Katherine Hand, of the Atomic Energy Project, University of California at Los Angeles, for the construction of the parabolic filter. The center of the filter had a thickness of 1.7 mm. Cu; the edge, 0.5 mm. Cu.

† The actual dose rate within the exposure container itself was slightly less, being 14.1 r/min measured in air at the bottom of the box.

‡ Versene, Regular Powder, Braun Chemical Co., Los Angeles.

§ Rockland Rat Diet in meal form, A. E. Staley Manufacturing Co., Chicago, Ill.

the exception of smaller body weight, no significant external differences were observed grossly between non-irradiated and surviving X-irradiated mice on either of the diets employed.

At the time of the first X-ray exposure, the body weight for both irradiated and non-irradiated mice on the purified diet averaged 27.4 gm. After 120 and 150 days, surviving animals in these groups averaged 26.5 and 38.8 gm. and 27.8 and 41.3 gm. for irradiated and non-irradiated mice, respectively. On the stock ration, irradiated and non-irradiated mice averaged 27.9 gm. at the time of the first X-ray exposure, and surviving aimals in these groups 25.7 and 34.2 gm., respectively, 120 days thereafter.

Time of onset of lesions.—In surviving X-irradiated animals, a latent period of several months preceded the appearance of periodontal pathology. Thus, in animals sacrificed 80 days following the first X-ray exposure and in irradiated animals which had succumbed during this period, no evidence of periodontal involvement was noted. These mice were essentially comparable to non-irradiated animals of the same age.

On the other hand, virtually all animals sacrificed 100 days after the first X-ray exposure and fed the purified diet exhibited at least minimal periodontal pathology. By 120–50 days after the first exposure, these lesions were moderate to severe in their extent in animals on the purified diet. Animals on the stock ration, however, revealed no evidence of periodontal involvement at 120 days. Finally, by 300 days, periodontal lesions in irradiated animals on the purified diet had become extensive and were frequently associated with exfoliation.

Gross findings.—In irradiated animals on the purified diet the most striking gross finding was the presence of a soft, friable, yellow-white mass in the gingival sulci, accompanied by some thickening of the adjacent gingival tissues. This adherent material was shown microscopically to consist of a population of mixed bacteria and fungi, together with food debris and inflammatory exudate, and will be referred to hereafter as the "plaque."

Initial lesions of the gingival sulcus appeared most frequently at the lingual and mesial aspects of the first maxillary and mandibular molars, followed frequently by the linguo-distal aspect of the third molars of both jaws. In an occasional animal, only a single tooth was involved, while, in others, only one quadrant was involved. As the severity of the lesion increased, however, the circumference of the gingival margins of all the teeth usually became obliterated, and the plaque extended onto their occlusal surfaces (Fig. 5, ***right***).

With increasing severity of involvement, the gingival sulcus became grossly deepened, and the soft tissues of the gum became flaccid and withdrawn from the necks of the teeth (Fig. 5, ***right***). The invasion of the sulcus by the plaque ultimately reached a point at which it extended virtually to the root apex, involving the interradicular zone as well. The most severely affected of these teeth were free of firm connection with bone or soft tissue and could be easily removed from the jaws by gentle traction. The ultimate expression of the periodontitis was exfoliation, and many samples were observed in which portions of teeth or whole teeth had been lost. In their place remained a soft pocket of the plaque, which could be followed with a probe to the bottom of the remaining alveolar socket. In a few instances, quadrants were found which were completely edentulous but in which healing had occurred. In none of the gross

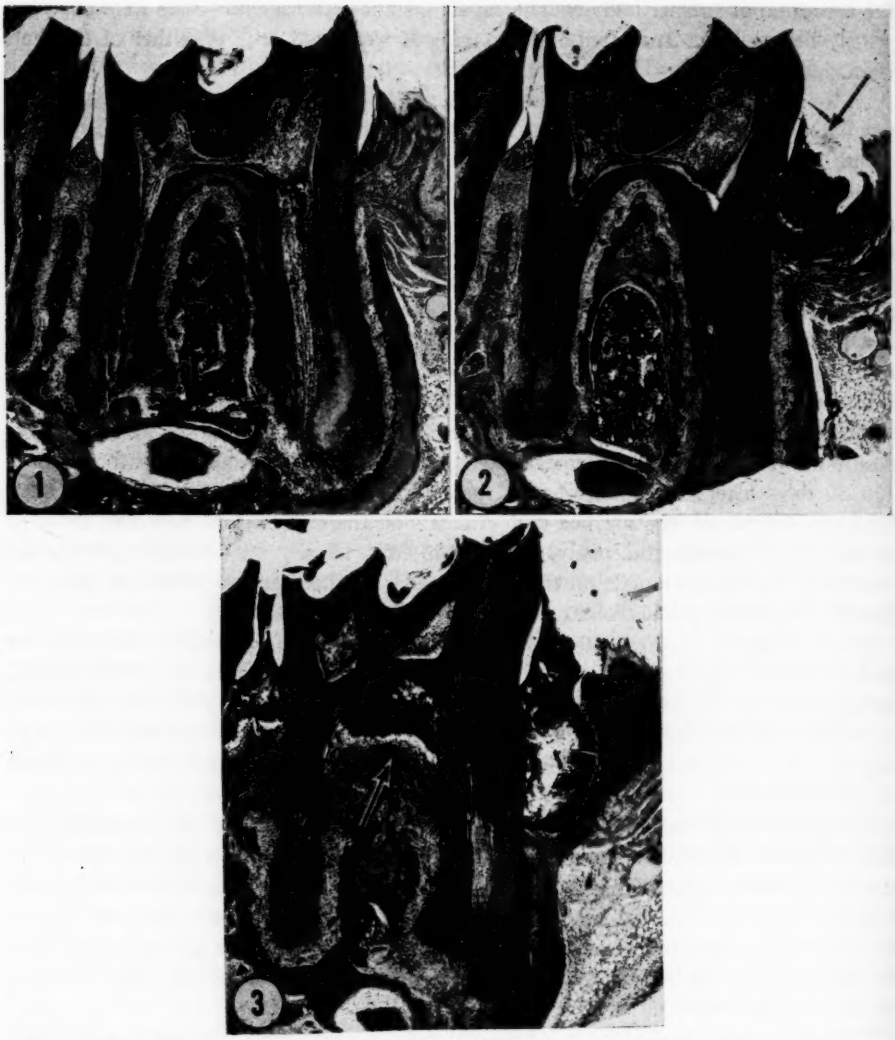

FIG. 1.—Mesiodistal section of mandibular first molar of a normal non-irradiated mouse at the age of 163 days. This animal is a control to that illustrated in Fig. 2. No lesions are apparent at gingival margin or in tooth proper. (Hematoxylin-eosin, Orig. Mag. ×35.)

FIG. 2.—Mesiodistal section of mandibular first molar from irradiated mouse sacrificed 100 days following the first X-irradiation. An early periodontal lesion (*arrow*) may be seen encroaching upon the gingival sulcus at the mesial aspect of the tooth. The tooth appears otherwise normal. (Hematoxylin-eosin, Orig. Mag. ×35.)

FIG. 3.—Mesiodistal section of mandibular first molar from irradiated mouse sacrificed 200 days following the first X-irradiation. Invasion of the bacterial plaque encroaches deep into gingival sulcus, also involving the interradicular area. Arrow indicates the extent of the interradicular lesion and the fact that its floor is covered by a relatively normal epithelium. Alveolar bone loss is well demonstrated. (Hematoxylin-eosin, Orig. Mag. ×35.)

samples was marked evidence of hemorrhage observed, nor were calculus deposits found to be a significant factor in the lesions.*

In contrast to the findings above, molar quadrants from non-irradiated animals and from irradiated animals on the stock diet showed little evidence of pathology. The three teeth in each were clean and firmly placed in their sockets, and the confirmation of their occlusal surfaces was usually easily discerned. Surrounding gingival tissues were firm and were closely applied to the margins of each tooth (Fig. 5, *left*). Occasionally, the gingival sulci and occlusal surfaces were obscured to some degree by the accumulation therein of amorphous debris. In a few control animals, the latter process had become so extensive as to resemble superficially the serious periodontal lesions

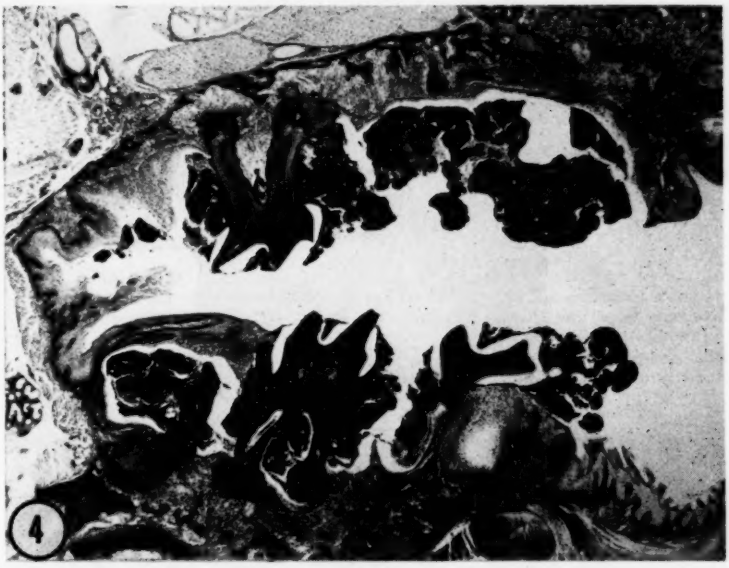

FIG. 4.—Parasagittal section through molar dentition of irradiated mouse sacrificed 300 days following first X-irradiation. Invasion of periodontal structures is most obvious. Exfoliation is a characteristic finding in animals of this age. The remaining teeth may be seen to be supported only at their root apices. (Hematoxylin-eosin, Orig. Mag. ×15.)

which characterized irradiated jaws. Subsequent microscopic examination of these teeth, however, did not demonstrate periodontal pathology.

Microscopic findings.—On the purified ration, the sequence of events observed microscopically in the irradiated mice followed the general pattern of development of primate periodontitis. Initial plaque formation at the gingival sulcus was followed by its progressive encroachment into the depths of the sulcus, then by degeneration of the periodontal connective tissues and alveolar bone, and, finally, by exfoliation of the teeth. This over-all pattern is well demonstrated in Figures 1–4, which illustrate the progress of the postirradiation lesion over a period of approximately 300 days, by which time exfoliation is common. However, the progress of these lesions differs in

* A number of animals, in both the X-irradiated and non-irradiated groups on the purified diet exhibited mild occlusal caries. These were not observed in animals fed the stock ration.

several respects from that of primate lesions in terms of its microscopic features. The material which follows emphasizes these differences within the over-all pathogenetic sequence and will be presented according to the nature of the tissues which are involved.

In the single series of X-irradiated mice on the stock ration, few, if any pathologic changes were observed.

Gingival tissues: Figures 1 through 3 indicate that the initial lesion following irradiation was one which first impinged upon the gingival sulcus (Fig. 2, *arrow*). Response of the gingival epithelium to the plaque was minimal. An inflammatory reaction of the subjacent periodontal tissues caused infiltration of the gingival epithelium by leukocytes, but there was no appreciable hyperplasia of the epithelium, a fact which

FIG. 5.—Gross appearance of maxillary molar quadrants of normal (*left*) and X-irradiated (*right*) animals. The animals were sacrificed at the age of 183 days. The relationship of the teeth and soft tissue in the control animal (*left*) is seen to be normal. Involvement of the irradiated quadrant (*right*) by bacterial plaque has caused separation of the teeth from their supporting soft tissues and loss of cuspal detail (120 days following first X-irradiation). The teeth in this severely affected jaw were very loose.

immediately differentiates this radiation-induced periodontitis in the mouse from primate periodontitis.

With continuing invasion toward the root apex and involvement of the underlying periodontal tissues, the gingival epithelium appeared to recede. Ultimately, the lesion attained a depth such that the interradicular zone was exposed, but, though the undersurface of the tooth was now involved by the plaque, the floor of the lesion was still formed by relatively normal gingival epithelium (Fig. 3, *arrow*).

During its initial invasion of the gingival sulcus, the plaque extended downward between the enamel and the epithelial attachment collar but left the enamel cuticle intact. In very seriously involved teeth, it was still possible to visualize the undisturbed enamel cuticle lying in close association to the plaque (Fig. 7; Fig. 8, *A;* Fig. 13).

FIG. 6.—Mesiodistal section of mandibular first molar from X-irradiated mouse sacrificed 200 days following first X-irradiation. Periodontal lesions in this animal had progressed to a somewhat greater extent than in that sample illustrated in Fig. 3, sacrficed at the same time. Involvement of mesial and interproximal aspects is striking. The interradicular zone also demonstrates periodontal and alveolar bone loss. Destruction of periodontal collagenous fibers is well demonstrated, particularly in interradicular zone. Close examination also reveals destruction of cementum along most of the length of the roots. (Mallory's triple stain, Orig. Mag. ×35.)

FIG. 7.—Mesiodistal section of maxillary first molar from X-irradiated animal sacrificed 120 days following first X-irradiation. Initial invasion of bacterial plaque between cementum and dentin is clearly visible. Note that gingival epithelium, although infiltrated with inflammatory cells, shows no evidence of hyperplasia. (Hematoxylin-eosin, Orig. Mag. ×160.)

Periodontal ligament and alveolar bone: It has already been noted that the periodontal lesions resulting from irradiation involve a dissolution of the periodontal ligament and alveolar bone. Within the periodontal supporting tissues at the earliest stages of invasion of the plaque, there was a moderate infiltration by inflammatory cells, particularly directly beneath the overlying epithelium. This infiltration never became particularly heavy in the depths of the periodontium. Neither were hyperemia, edema, or hemorrhage striking features of the lesion (Figs. 7, 10, and 11).

Continuing encroachment of the plaque down along the margin of the tooth was accompanied by lytic changes in the periodontal collagen bundles (Fig. 6). Simultaneously, the normal cellular population of the periodontal tissue became necrotic

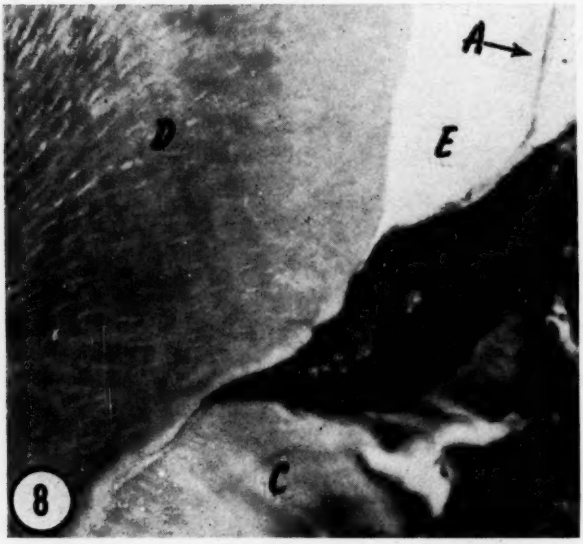

FIG. 8.—Detail of section similar to that illustrated in Fig. 7. *A*, enamel cuticle; *C*, cementum; *D*, dentin; *E*, enamel space. A close relationship between enamel surface, dentin, and cementum exists in the cervical region. The bacterial plaque invades along the enamel cuticle (*A*), finally coming between it and the overlapping cementum (*C*). Thereafter, cementum is progressively peeled away from the surface of the dentin. (Hematoxylin-eosin, Orig. Mag. ×550.)

and in well-developed lesions could be seen to consist of pyknotic and fragmenting nuclear remnants, together with some inflammatory cells and macrophages (cf. Figs. 10 and 11). In all the material observed, no evidence of bacterial invasion directly into the substance of the periodontal ligament was noted, e.g., no true abcess formation occurred. Thus, while the gingival epithelium apparently resisted invasion by the plaque, collagenolytic and cytotoxic factors from the plaque penetrated the epithelium and destroyed the collagenous tissues below.

Dissolution of alveolar bone followed closely the loss of periodontal fiber integrity and may be appreciated in the accompanying illustrations (Figs. 1–4, 6). Careful observation of areas of bone destruction revealed that it was mediated at least in part by the activity of osteoclasts, although these cells were not especially prominent in the preparations. Despite the fact that massive alveolar bone resorption ultimately

occurred in these lesions, the bone always appeared to maintain the capacity for regeneration, since functional osteoblasts could be identified on the surface of the bone opposite to that which was being resorbed.

Enamel, dentin, and cementum: Examination of the response of the mineralized dental tissues to increasing degrees of involvement in the periodontal lesion revealed that dentin and cementum were particularly affected, while enamel was most probably resistant. Since the preparations utilized for microscopic study were demineralized, any assessment of enamel involvement was, of necessity, inferential. However, the lack of gross lesions in the enamel prior to decalcification, together with the integrity of the enamel cuticle and over-all shape of the enamel spaces in severely involved demineralized specimens, combine to suggest that the enamel was little affected by the lesion (Figs. 7 and 13).

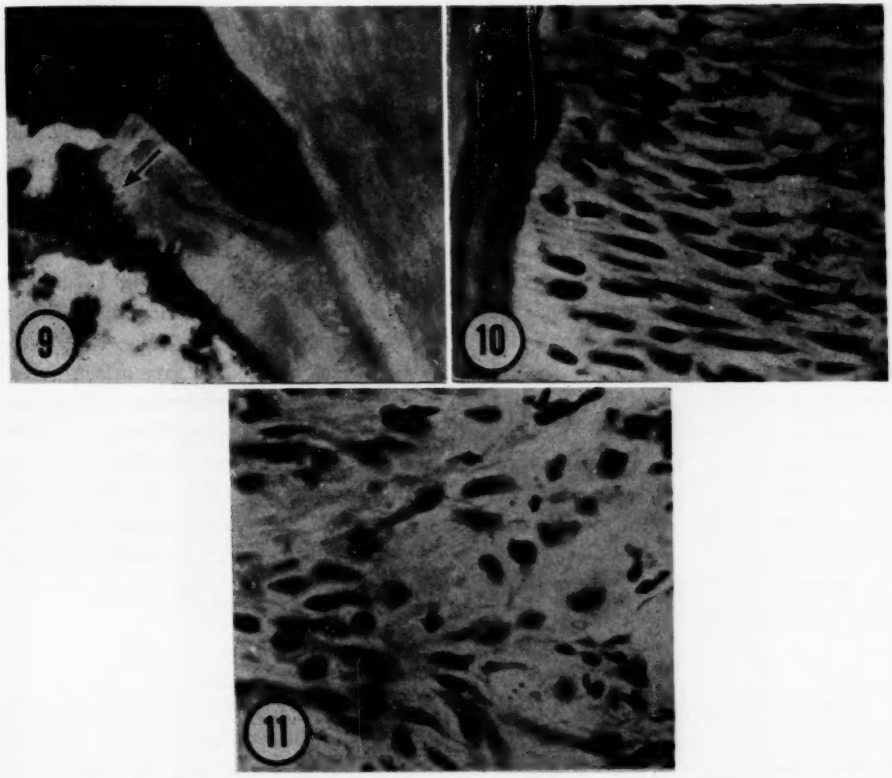

FIG. 9.—Detail of plaque invasion of cemento-dentinal junction at a slightly more advanced stage than illustrated in Fig. 8. Separation of cementum from dentin along the cemento-dentinal junction is clearly apparent. Invasion of bacterial material into spaces corresponding to Sharpey's fibers of cementum is indicated by the arrow. (Hematoxylin-eosin, Orig. Mag. ×550.)

FIGS. 10 AND 11.—Comparison of normal periodontal architecture (Fig. 10) and periodontal tissues in X-irradiated animal, sacrificed 120 days following first X-irradiation (Fig. 11). Cell destruction and collagenolysis, accompanied by loss of normal fiber orientation, are characteristics of the X-irradiated series at this age and are in sharp contrast to normal periodontal features. (Hematoxylin-eosin, Orig. Mag. ×550.)

Cementum was progressively necrotized and eroded as the periodontal lesion became more severe, another feature distinguishing this lesion from the primate form of periodontitis. As the gingival margin receded with increasing penetration of the plaque, the plaque was brought into contact with the cemento-enamel junction. In the majority of the specimens examined, the cementum normally overlapped the enamel in the cervical region. Migration of the plaque downward, closely abutting on the enamel cuticle, finally brought the plaque into a position that permitted it to invade between the overlapping cementum and the cervical enamel (Fig. 7). Thereafter, the cementum was progressively freed from its connection to the dentin, as the process of invasion by the plaque continued (Figs. 8 and 9). Concomitantly, bacterial invasion of the cementum proper appeared to occur along pathways originally occupied by Sharpey's fibers (Fig. 9, *arrow*). Ultimately, cementum was broken down, and in well-developed lesions the margin of dentin was often completely freed of a cemental covering (Fig. 6).

Removal of cementum created a close apposition between the plaque and the dentin and allowed direct bacterial invasion of the dentinal tubules (Figs. 3 and 12). Pulpar necrosis invariably followed dentinal involvement. Dentinal necrosis and resorption of the affected tooth then followed as a characteristic pathologic feature (Fig. 12) and ultimately led to exfoliation. However, it is of interest to note that occasional teeth were involved in the lesion only in part. In these, the lesion had virtually destroyed the periodontal attachment and root system on one side, without encroaching upon another portion of the same tooth (Fig. 12).

Another interesting and unique feature of these lesions is illustrated in Figures 12 and 13. Here and in many other equally severely affected teeth, there had been a "recurrent" invasion by the plaque along the dentino-enamel junction (Fig. 12, *circle*). The invasion appeared to be restricted to the dentin and seemed to have arisen by extension of the original plaque from its point of contact with cementum, dentin, and enamel at the cervical region. Careful observation of the "recurrent" lesion revealed that it was limited in its extent superficially by the dentino-enamel junction and on its deep aspect by what is interpreted to be a limiting membrane (Fig. 13). Neither the composition nor the significance of the latter structure is understood. Again, resistance of the enamel to the effects of the bacterial plaque was implied by the maintenance of the general morphology of the enamel space and enamel matrix (Fig. 13, *E*), even in this "recurrent" lesion.

DISCUSSION

Somatic radiation damage and possible measures for protecting against it have become the object of heightened experimental interest during recent years. Current reviews[4] indicate that progress in this area has been rapid and may be expected to expand even further in the future. However, of the considerable body of information which is now available in the literature regarding somatic radiation effects, only a very small part is concerned with the oral cavity, and this deals either with acute radiation death of the organism or with the effects of single, and frequently lethal, exposures to X-ray.[5–7]

Fundamental differences in the response of the body to radiation, depending on the manner in which the dose is delivered, i.e., dose rate, are well recognized.[8, 9] This

phenomenon constitutes a major area of study, but, as was noted above, only within the past year has its importance been suggested in terms of the oral cavity.[1, 2]

It seems certain that the periodontal lesions described above have arisen as the result of the course of fractionated whole body X-irradiation to which the experimental animals were subjected. This type of lesion has not been described as a sequela of single exposures to X-irradiation, either minimal or massive. Spontaneous periodontal pathology has been described previously, however, in the non-irradiated mouse[10, 11] and other related species.[12, 13] Invariably, development of such lesions seems to depend on several factors, usually in combination, including heredity, mechanical trauma, age, and diet. In the present studies, heredity was a constant factor, and so too, presumably, was mechanical trauma. The latter, however, may be regarded as a contributory factor, since the constant stresses of mastication on an already diseased periodontal apparatus must certainly add to the severity of the process. In view of the apparent interplay between X-irradiation, diet, and age in the present study, it

FIG. 12.—Mesiodistal section of mandibular first molar from X-irradiated animal sacrificed 180 days following first X-irradiation. Extensive invasion of bacterial plaque into mesial and interradicular aspects of tooth is clearly evident. Conversely, the interproximal area (*left*) appears to be uninvolved. Destruction of the mesial root is well advanced. The area inclosed by the circle illustrates the "recurrent" invasion of the dentino-enamel junction by the lesion. (Hematoxylin-eosin, Orig. Mag. ×55.)

seems appropriate to attempt to derive some functional basis upon which the development of these lesions may be explained. Two manifestations of radiation injury—namely, diminution of body resistance to infection and the so-called "aging" effect of radiation—appear pertinent to this discussion.

Lowered resistance to a wide variety of infective agents following whole body X-irradiation is a well-established finding. The initial invasion of the plaque and its subsequent destructive course would tend to support the concept that natural resistance to this type of infection had been markedly reduced. In addition, it is not beyond the realm of possibility that alterations in the bacterial flora of the oral cavity, consequent to irradiation, may have contributed to the development of periodontitis. It would be of great interest to investigate the properties of the plaque by analytical microbiologic methods.

There is some indication based on preliminary histologic studies of the present material that salivary function, particularly of the palatal gland system, may have been impaired. Areas of fibrosis and reduced glandular tissue were prominent in several irradiated animals on the purified diet. If confirmed, this observation would indicate a

FIG. 13.—Detail of the dentino-enamel junction from tooth similar to that illustrated in Fig. 12, showing "recurrent" invasion by plaque. *D*, dentin; *E*, enamel space, containing some residual enamel matrix. The contours of the enamel space remain normal despite involvement of the dentino-enamel junction by the invading plaque. The enamel cuticle remains intact. The lesion is limited at the surface of the enamel by the cuticle, and at the dentino-enamel junction by the junction itself. On its deep aspect in the dentin the lesion appears to be limited by a membrane. (Hematoxylin-eosin, Orig. Mag. ×550.)

more specific type of radiation-induced somatic lesion, with its focus upon the oral environment. The major cervical glands, however, have not yet been seen to exhibit evidence of injury. Impaired salivary function could play an important etiological role in the lesions observed, by facilitating the accumulation of plaque material.

Spontaneous periodontal lesions in inbred strains of mice[10, 11] appear only after the animals have lived for over 1 year. It is impossible to say with certainty that the control animals of the present study would not have developed lesions of this type, had they been allowed to live longer. In view of the "aging" effect of radiation, it is interesting to speculate upon the early appearance and relatively rapid development of the periodontal lesion in our irradiated animals on the purified diet. Perhaps these lesions represent a speeding-up or accentuation of normal aging processes.

It is also of interest that such lesions did not occur in X-irradiated mice on the stock ration. All surviving mice in this group, however, were sacrificed 120 days after the first X-ray exposure, and it is possible that lesions might have occurred, had the animals been permitted to survive longer. The stock ration, therefore, may merely have delayed, rather than prevented, the occurrence of such lesions. Further studies are indicated to determine the factor (or factors) in the stock diet responsible for this effect.

SUMMARY

Studies were made of the effects of multiple sublethal doses of total body X-irradiation (6 weekly exposures of 200 r each) on the subsequent appearance of the periodontal and dental tissues of male mice fed either a highly purified diet complete in all known nutrients or a natural food stock ration.

Periodontal lesions were observed in X-irradiated animals on the purified diet at 100 days following the first irradiation exposure, but not prior to this time. The subsequent development of the lesions generally paralleled that of primate periodontitis and was characterized by plaque formation, gingival recession, periodontal destruction, dental necrosis, and exfoliation.

In contrast to findings on the purified diet, no periodontal pathology was observed in X-irradiated mice on the stock diet.

The multiple sublethal mode of exposure to total body X-irradiation appears to be a primary etiologic factor in the formation of periodontal lesions in mice fed the purified diet. Secondary factors, such as age, diet, trauma, oral bacterial flora, heredity, etc., may also be implicated. The possible significance of such factors is briefly discussed.

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Synthesis and Secretion of Amylase by Rat Parotid Gland Slices

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Synthesis of protein by animal tissues in vitro has been studied in a number of ways.[1–4] Most of them involved the inclusion of labeled amino acids in proteins or substances said to be proteins because of their precipitability or other chemical reactions.

Hokin[5] approached the problem from another point of view. He showed that slices of pancreas were able to synthesize amylase enzyme in vitro. This enzyme has a considerable protein content. This is the basis upon which Hokin concluded that pancreas tissue slices in fact synthesized protein under certain well-defined conditions of nutrition and gaseous atmosphere. He further demonstrated the effects of certain inhibitors upon amylase synthesis by the slices. Most interesting of all, he showed that carbamylcholine, acetylcholine, and pilocarpine influenced the *secretion* of amylase by the pancreas slices into the surrounding medium.

Hokin's findings stimulated our interest in the possibility of demonstrating the synthesis of amylase and other enzymes, or hormones, by slices of parotid glands. The possible effects of two oppositely acting drugs, pilocarpine and atropine, on the synthesis and secretion of amylase by the salivary gland were also considered. It is known that in vivo, atropine produces a thick, sparse saliva, while pilocarpine produces a thin, copious flow.

The objectives of this study, then, were two: (*a*) to study the *synthesis* of amylase by parotid gland slices in vitro under several conditions of incubation and (*b*) to study the effects of pilocarpine and atropine upon the *secretion* of amylase by the parotid gland slices in vitro. It was hoped thereby to obtain information on the mechanism of amylase synthesis and the process involved in the secretion of enzymes and other proteins by an exocrine gland such as the salivary gland.

EXPERIMENTAL METHODS

Preparation and incubation of parotid gland slices.—Sprague-Dawley rats were used for all the experiments. The animals were fed Rockland Small Animal diet, ad libitum, and supplied with adequate tap water.

One hour prior to killing the animals, 0.2 mg. carbamylcholine per kg. body weight of rat was injected intramuscularly. Salivation usually began in 3–5 minutes. This treatment was found to be desirable, in preliminary experiments, in order to deplete

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the glands to a reproducible minimum. When this pre-treatment was omitted, the basal levels of parotid gland amylase fluctuated over a wide range.

The animals were killed by a sharp blow which broke the neck. Etherization or injection of pentobarbital was tried in preliminary trials but was found to affect synthesis by the glands adversely.

The parotid glands were immediately removed and placed in glass dishes kept cold in an ice bath. The lobes were freed as much as possible from connective tissue. Slices approximately 0.5 mm. were cut with a mechanical slicer. The slices were immediately chilled and kept cold in a covered Petri dish on filter paper wetted with Krebs-Henseleit solution.[6]

When a sufficient number of slices had been prepared, they were weighed and placed in respirometer* reaction vessels. The slices ranged in weight from 60 to 100 mg. In each experiment, unincubated weighed slices were immediately placed in a drying oven at 105° C. and dried for 24 hours. They were again weighed. This was considered to be the dry weight upon which the activity of the slices was based. It was assumed that the ratio of dry-to-wet weight was constant for each slice of a particular gland. This is substantially accurate because the usual ratios deviated only slightly from 0.265. Five ml. of media were added to the outer well of the flask and kept in a tray of chopped ice until all the vessels were prepared, in order for all the flasks to begin their incubation at the same time. Usually less than 45 minutes elapsed from the killing of the animal until the start of incubation. The flasks were incubated with shaking for the times indicated in the tables, at 37.5° C. In some of the trials, the flasks were gassed to establish the appropriate atmosphere.

Media.—Three media were used for the experiments in preliminary trials. *Medium A* consisted of the bicarbonate saline of Krebs and Henseleit[6] gassed either with 5 per cent CO_2 and 95 per cent O_2 or 5 per cent CO_2 and 95 per cent N_2. To 100 ml. of this solution were added 20 mg. of each of the following amino acids: *L*-arginine, *L*-aspartic acid, *L*-citrulline, *L*-cysteine, *L*-glutamic acid, *L*-glutamine, glycine, *L*-histidine, *L*-hydroxyproline, *L*-isoleucine, *L*-leucine, *L*-lysine, *L*-methionine, *L*-phenylalanine, *L*-proline, *L*-tryptophane, *L*-tyrosine, and *L*-valine, together with 40 mg. each of *DL*-serine and *DL*-threonine. Two hundred mg. of glucose were added.

Medium B consisted of Krebs-Henseleit bicarbonate saline[6] containing 0.2 per cent glucose and 0.4 per cent of casein hydrolysate (Matheson Coleman and Bell) to which 2 parts of *L*-tryptophane per 100 parts of casein were added. This medium also was gassed either with 5 per cent CO_2 and 95 per cent O_2 or 5 per cent CO_2 and 95 per cent N_2.

Medium C consisted of Krebs-Henseleit bicarbonate saline only. For those experiments where pilocarpine or atropine was used, these substances were added to the incubation mixture to make the final concentration of 0.1 mg. per 100 ml.

Treatment of tissues after incubation.—After the 2-hour incubation period, the Warburg respirometer vessels were returned to a tray of chopped ice.

Hokin[5] has shown that when pancreas slices are incubated with suitable media, some of the total amylase activity is found in the tissues, while some of it can be demonstrated in the surrounding medium. He suggested that the amylase level in the medium represents that which had been formed in the tissue and then liberated into

* Warburg.

the medium. Such liberation may be the result either of secretion or of simple diffusion. Subsequent studies allow us to differentiate between the two processes.

It is evident that the amylase in the medium is not the result of synthesis in the compartment, because there is no synthesis in the medium in the absence of tissue nor is there synthesis of amylase if the tissue is homogenized in saline buffer before incubation.

Consequently, in this study the amylase activity in both the tissue and the medium was determined. We assumed that an increase in the sum of these activities, referred to as the "total" amylase, represents that which was synthesized. The increase in the activity of the "medium" amylase level represents that which was synthesized and transferred, without, at the moment, deciding whether the transfer was passive or truly secretory.

TABLE 1

VARIATIONS IN AMYLASE ACTIVITY OF FRESH SLICES FROM A SINGLE PAROTID GLAND

Dried Wt. (Calculated Mg.)	Amylase Units per Mg. Dry Wt.	Dried Wt. (Calculated Mg.)	Amylase Units per Mg. Dry Wt.
15.0.....	107	58.4.....	112
27.2.....	105	107.8.....	106

The slices were removed from the vessels with forceps and blotted with filter paper. One-ml. portions of the fluid in the vessel were diluted by adding either 10, 15, or 20 ml. water and kept refrigerated. These samples are subsequently referred to as the "medium." The tissue slices were separately ground in a mortar with 1 gm. of powdered glass and 5 ml. of ice water for 5 minutes while the mortar was kept chilled in an ice bath. The mixture was centrifuged at 3,000 rpm for 15 minutes. A clear supernatant resulted. One-ml. portions of the supernatant were diluted with 10, 15, and 20 ml. water, respectively. These solutions are hereafter referred to as the "tissue." All samples were stored in a refrigerator until assayed.

Methods of assay.—The Smith and Roe[7] method was used to assay amylase activity. Preliminary trials were run on each sample. The time of incubation of the final assay was adjusted so that the range of the method was not exceeded. This procedure is permissible because the digestion rate of starch over the standard 30-minute test period is linear. The starch-iodine color was measured on a spectrophotometer* at 620 millimicrons.

The activity of amylase in the tissue and medium were expressed in units as stated by Smith and Roe. The sum of the tissue and medium amylase activity is hereafter referred to as the "total" activity.

RESULTS

Reliability of amylase activity assay in slices.—The amylase activity of slices freshly prepared from different parts of the gland and cut to varying weights showed a remarkable constancy (Table 1).

* Coleman Junior Spectrophotometer.

Amylase determinations made on aliquots of homogenates from a single slice and performed in the presence of atropine or pilocarpine showed that these two drugs had no effect on the determination.

Increase in amylase activity on aerobic incubation.—Parotid gland slices, incubated aerobically in the media, as indicated in Table 2, clearly showed the synthesis of amylase. Since the synthesis was not too different whether Medium A or Medium B was employed, all subsequent tests were performed with only Medium B.

The data further show that considerable amounts of amylase were discharged into the medium during the first 30 minutes of incubation.

Effect of aerobic and anaerobic incubation on synthesis and secretion.—We determined the effects of the secretory stimulant, carbamylcholine, on synthesis and secretion both aerobically and anaerobically. When the gas phase was CO_2 and N_2, a pellet of phosphorus was kept in the center well of the reaction vessel to assure the absence of oxygen.

TABLE 2

RATE OF AMYLASE SYNTHESIS BY GLAND SLICES

	Incubation Time (min.)	Units of Amylase Activity per Mg. Initial Dry Wt. (Av. of 10 Trials Each)		
		Medium	Tissue	Total
Medium A	0	0	78±5	78±4
	30	20±2	62±3	92±5
	60	25±3	75±4	100±5
	120	30±2	82±5	112±6
	180	36±3	105±5	141±7
Medium B	0	0	68±4	68±4
	30	25±2	51±4	76±6
	60	28±3	66±3	94±4
	120	32±3	74±5	106±7
	180	38±2	97±5	135±8
Krebs-Henseleit carbonate-saline only	0	0	74±5	74±5
	30	12±2	66±4	78±5
	60	29±3	45±4	74±4
	120	41±4	47±3	88±6
	180	52±4	38±5	90±6

The protocol for these trials and the results are shown in Table 3.

The results show that the secretory stimulant, carbamylcholine, increased the fraction of amylase in the medium aerobically but not anaerobically. Furthermore, considerable total amylase was formed aerobically but not anaerobically.

The effects of atropine and pilocarpine on amylase synthesis and secretion.—The effects of these two drugs are dramatically shown in Table 4. The slices evidently synthesized more amylase in the presence of the secretory stimulant than in the presence of the secretory depressant. That this is not simply a matter of totally inhibiting amylase synthesis is shown by the fact that, while the medium activity increased only a little, the tissue activity increased far out of proportion.

DISCUSSION

We have shown that fresh rat parotid gland slices, incubated in vitro, could synthesize amylase when they were supplied with an external source of certain amino acids. The slight increase in amylase in the non-fortified carbonate saline could well have been due to amino acids becoming available through autolysis of the slices themselves. These tissues incubated in non-fortified carbonate-saline also showed an increase in medium amylase. This change probably did not represent true secretion but rather was passive transfer of tissue amylase made possible by tissue damage during slicing. The latter view is preferred because of the concomitant fall in tissue amylase, as the medium amylase rose. A similar early leaching of pre-existing amylase was also observed in the tissues incubated in Medium A and Medium B. After this initial period, there seemed to be a steady rise in activity in both the medium and the tissue. These findings suggest a true synthesis, *de novo*, of amylase, and not just a conversion of an amylase zymogen to active amylase. The latter would most likely also have taken place anaerobically, but that was not found to have occurred. Amylase activity was increased aerobically only.

The tissue slices in an aerobic atmosphere seemed actively able to secrete amylase as they synthesized it. Very little secretion was found to take place anaerobically.

TABLE 3

SYNTHESIS AND SECRETION OF AMYLASE UNDER AEROBIC AND ANAEROBIC CONDITIONS

Incubation Period (Hours)	Carbamylcholine (Mg/100 Ml)	Gas Phase	Units of Amylase Activity per Mg. Initial Dry Wt. (Calc.) (Av. of 10 Trials Each)		
			Medium	Tissue	Total
0			0	56±4	56±5
2	0	CO_2, N_2	15	43±3	58±4
2	1	CO_2, N_2	17	43±3	60±5
2	0	CO_2, O_2	24	91±6	115±6
2	1	CO_2, O_2	55	75±5	130±7

TABLE 4

EFFECT OF ATROPINE AND PILOCARPINE ON AMYLASE SYNTHESIS AND SECRETION

Incubation Time (Hours)	Drug	Units of Amylase Activity per Mg. Initial Dry Wt.		
		Medium	Tissue	Total
0		0	65±4	65±5
2		25±3	100±6	125±7
2	Atropine	5±4	98±5	103±6
2	Pilocarpine	58±5	73±6	131±7

Furthermore, the tissue concentration of amylase seemed to control the rate of its synthesis. This conclusion results from the observation that those slices which were allowed or encouraged to secrete their products in the presence of carbamylcholine or pilocarpine synthesized considerably more amylase than those whose secretion was diminished by the drug atropine. While this drug did not materially retard the tissue concentration, there resulted a substantially decreased total enzyme synthesis. These results suggest an equilibrium condition which seems to be the limiting factor in the rate of synthesis. This supposition is strengthened by the observation that the total activity of the pilocarpine-treated slices was greater than that of the atropine-treated ones, whereas the tissue activity was not in proportion. It may, of course, be that the secretory stimulus, pilocarpine, by depleting the tissue amylase concentration may have stimulated the rate of new enzyme formation. Our data do not allow us to differentiate between these alternate hypotheses.

In future work, we plan to test this matter further, as well as to study the synthesis and secretion of other products of parotid gland activity, by using the methods described herein.

SUMMARY

Parotid gland slices from rats were incubated in reaction vessels which contained media of known composition. After incubation, the medium around the slices as well as the slices themselves were assayed for amylase. The effects of the drugs atropine and pilocarpine were also studied by including them in the substrate for some of the experiments.

The results of the experiment allow us to draw the following conclusions: (1) Parotid gland slices from rats can synthesize amylase when incubated aerobically in the presence of an amino acid source. (2) The slices can secrete amylase into the surrounding medium. (3) The secretory stimulant, pilocarpine, can increase the in vitro secretion of the gland slices. It also seems to bring about a net increase in amylase synthesis. (4) The secretion depressant drug, atropine, can decrease the secretion of amylase in vitro by gland slices. It also seems to bring about a net decrease in amylase synthesis.

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Epithelial Activity in the Exfoliation of the Deciduous Tooth and the Emerging Permanent Tooth

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The relationship of the gingival sulcus of the deciduous tooth to its permanent successor has not been described. This study describes the sequence of changes which occur and establishes that the gingival sulcus epithelium does not participate in the formation of the sulcus of the permanent tooth.

MATERIALS AND METHODS

Two *Macacus rhesus* monkeys were used because their dentition is similar to the human. The incisor teeth had either just emerged or were about to emerge. Sections of the mandible and maxilla were fixed in 10 per cent formalin for 20 days. During the following 21 days, the specimens were decalcified in 5 per cent nitric acid. The solution was changed daily. After decalcification they were washed in running water for 29 hours. The specimens were then placed in progressively more concentrated ethanol baths for 6 days. After remaining in absolute ethanol for 24 hours, the ethanol was replaced with benzene. Paraffin was then substituted for the benzene. The resulting blocks were sectioned labiolingually at 10 μ. All specimens were stained with hematoxylin and eosin.

RESULTS

The pattern of resorption of the deciduous tooth crown followed several forms. It was directly related to the position that the erupting permanent tooth took to its predecessor. The specimens in this study showed three variations: (1) the permanent tooth erupting directly beneath the deciduous tooth; (2) the permanent tooth erupting lingually to the deciduous tooth; and (3) the permanent tooth erupting labially to the deciduous tooth. Because all the specimens in this study were sectioned labiolingually, variations in a mesiodistal direction were not included.

Centric eruption.—When the permanent tooth erupted directly underneath the deciduous tooth, the resorption and exfoliation of the latter occurred harmoniously. Figure 1 shows a maxillary permanent lateral incisor with its deciduous predecessor *in situ*. The bony crypt had resorbed in the incisal area as the permanent tooth erupted toward the oral cavity. The presence of Howship's lacunae indicated that resorption

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was still active when the specimen was taken. The enamel was lost in decalcification.

There was a layer of degenerated ameloblasts over the incisal area of the permanent tooth (Fig. 2). Directly over the degenerating ameloblasts, the stratum intermedium proliferated markedly. The remaining ameloblastic layer retained its columnar form. At this stage, there was no continuity between the enamel epithelium of the permanent tooth and the sulcus epithelium of the deciduous tooth or the oral mucosa.

Figure 2 also shows that the stratum intermedium had proliferated into the young vascular connective tissue underneath the resorbing deciduous tooth. Here the connec-

FIG. 1.—Permanent lateral incisor almost ready to emerge. The crown of the deciduous tooth is displaced lingually.

tive tissue was bordered by osteoclasts resting in Howship's lacunae in the dentin. On the labial surface there was no sulcus epithelium, and the oral mucosa approximated the resorbing dentin. On the lingual (Fig. 2), the deciduous odontogenic epithelium had proliferated toward the dense inflammation in the corium and migrated around the fundus of the sulcus toward the inner resorbing surface of the dentin. The cementum at the cervix of the deciduous tooth showed neither periodontal membrane nor epithelial attachment.

Exfoliation of the deciduous tooth.—At the time of exfoliation (Fig. 3), a layer of epithelium had migrated over the connective tissue surface. The arrow points to a small ulcerous area due to incomplete epithelialization. It was difficult to tell the origin of this covering epithelium, but it possessed budding characteristics suggestive of

odontogenic epithelium. It lacked both a granular layer and a keratinized layer. At the periphery (Fig. 4), the masticatory mucosa (*MM*) proliferated over the surface of this initial covering epithelium.

Figure 5 shows a later stage. The concavity had repaired, and the surface was completely keratinized by the covering oral epithelium. There were normal rete pegs on both the labial and the lingual. In the area of the exfoliated deciduous tooth, how-

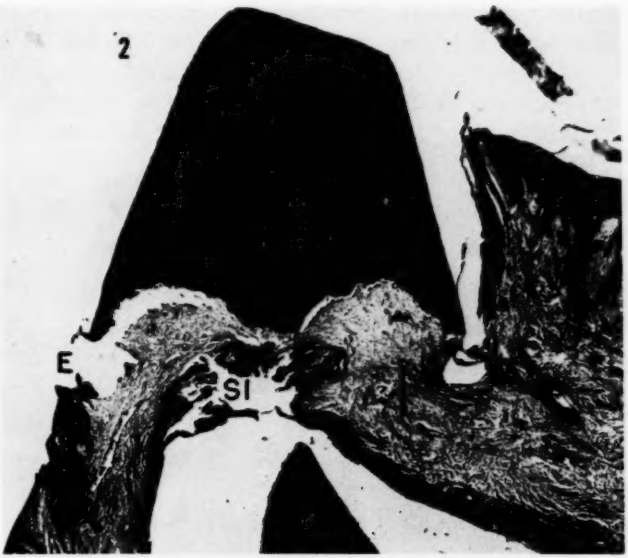

FIG. 2.—High magnification of resorbing deciduous tooth in Fig. 1. The proliferating stratum intermedium (*SI*) is separated from resorbing dentin by a layer of connective tissue.

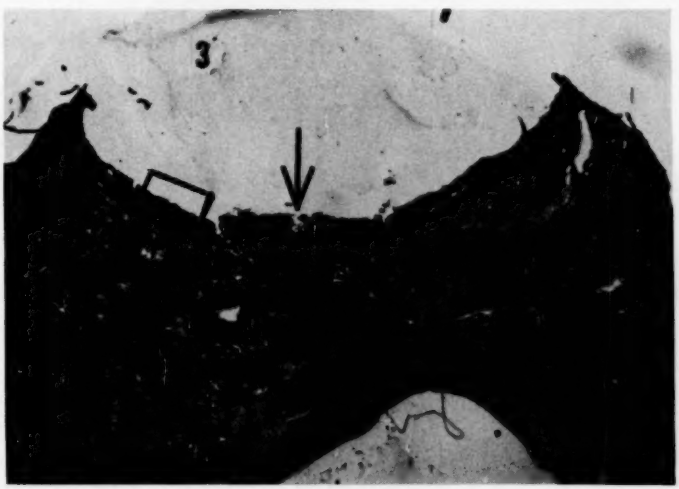

FIG. 3.—Area of ridge after exfoliation of the deciduous tooth. Note migration of the epithelium. Arrow points to a small ulcer.

ever, the rete pegs were short. An area of proliferating odontogenic epithelium, characterized by its budding growth, lay beneath the repaired surface. Two epithelial rests (*ER*) were found in the area.

At a later stage the surface was completely repaired with keratinized oral mucosa. The epithelium of odontogenic origin could not be differentiated.

Figure 6 shows the relationship of the oral mucosa to the odontogenic epithelium of

FIG. 4.—High magnification of the box in Fig. 3. Shows masticatory mucosa (*MM*) migrating over the odontogenic epithelium (*OE*).

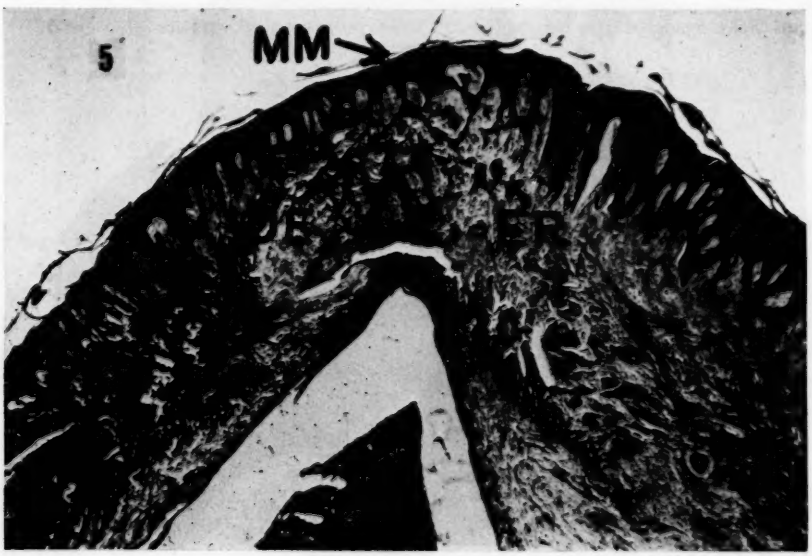

FIG. 5.—Masticatory mucosa (*MM*), odontogenic epithelium (*OE*), epithelial rests (*ER*)

the permanent tooth after emergence. At the margin of the gingiva, under higher magnification (Fig. 7), we can distinguish that the masticatory mucosa migrated behind the odontogenic epithelium to its normal relationship.

In centric eruption, the ultimate fate of the sulcus epithelium is to aid in the repair of the defect caused by the exfoliation of the deciduous tooth. It is then replaced by keratinized oral mucosa. When the permanent tooth emerges, the oral mucosa joins with the odontogenic epithelium of the permanent tooth to form the new sulcus.

Eccentric eruption.—When the permanent tooth erupted either labially or lingually, the pattern of resorption of the deciduous tooth conformed to the eccentric relationship. If the permanent tooth erupted toward the labial, the resorption of the deciduous tooth was most advanced on the labial (Fig. 8). If it erupted toward the lingual, the resorption of the deciduous tooth was most advanced on the lingual (Fig. 9). There

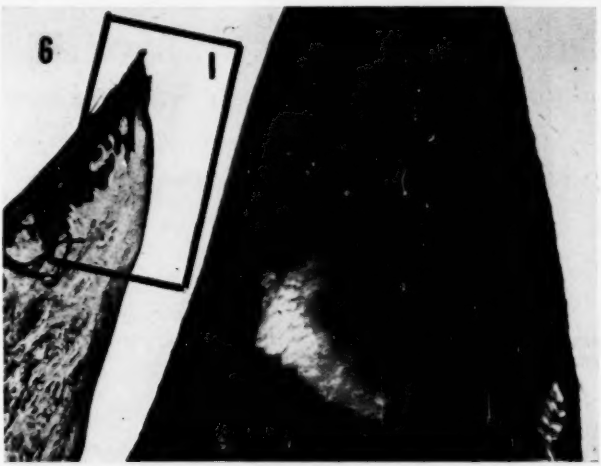

FIG. 6.—Low magnification of emerged permanent tooth, showing the relationship of the oral mucosa to the odontogenic epithelium.

may or may not be periodontal membrane attached to the cementum on the less resorbed side.

There was however, additional cellular activity associated with other changes, in contrast to the general picture of centric eruption. In eccentric eruption, the odontogenic epithelium of the permanent tooth showed the same changes as have been described for centric eruption. The marked difference was the proliferation of the stratum intermedium cells in the incisal area.

In every instance of eccentric eruption, tooth fragments, some containing cementum and dentin, others with dentin alone, were found in the area of the resorbing deciduous tooth. The cells of the stratum intermedium of the permanent tooth proliferated to envelop the fragments (Fig. 10). Eventually most, if not all, of the fragments were encapsulated by epithelium. Even when the exposed portion of the deciduous tooth had been exfoliated, fragments either completely or partially enveloped by the epithelium remained *in situ* (Fig. 11). It is difficult to establish whether the fragments resulted from resorption or fracture. Figure 10 shows resorption which almost per-

FIG. 7.—High magnification of box in Fig. 6. Shows masticatory mucosa (*MM*) moving apically behind the odontogenic epithelium (*OE*).

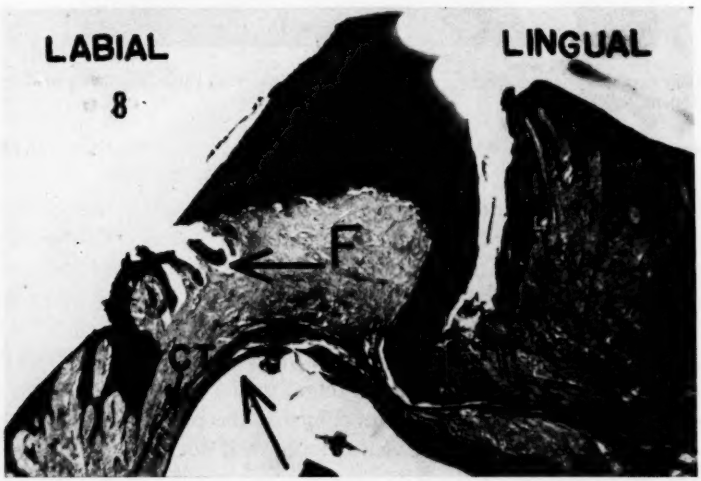

FIG. 8.—Labiolingual section of permanent tooth erupting toward the labial. Arrow shows direction of eruption. Note band of connective tissue (*CT*) over erupting permanent tooth and fragments (*F*).

FIG. 9.—Labiolingual section of permanent tooth. Arrow shows direction of eruption toward the lingual.

FIG. 10.—Arrow shows resorption of the deciduous tooth almost to perforation. Fragment (*F*) shows Howship's lacunae above and to the left. On the right, a fractured surface.

forated the cementum. At another site a fragment appeared to have been fractured. Other areas of the same fragment showed Howship's lacunae.

In Figure 12, the deciduous tooth persisted after the emergence of its successor. The microscopic anatomy of the sulcus showed columnar epithelium almost in contact with the oral mucosa, establishing that the sulcus of the permanent tooth was formed by oral mucosa and the odontogenic epithelium of the permanent tooth.

On the labial of the deciduous tooth (Fig. 13), the sulcus epithelium, rather than migrating toward the odontogenic epithelium of the permanent tooth, migrated along the resorbed inner surface of the crown of the deciduous tooth.

FIG. 11.—Showing proliferation of the stratum intermedium cells surrounding fragments of the deciduous tooth. Inflammatory cells in area (*I*).

DISCUSSION

Aisenberg,[1] in his description of the relationship between the erupting permanent incisor and its deciduous predecessor, described both a lingual and an apical relationship. At least in the monkey, a labial relationship must be added. In all instances the pattern of resorption of the deciduous tooth conformed to the position of its successor. This would suggest that the pressure of the erupting tooth on the connective tissue determined the pattern of resorption. Pressure, however, is a physical phenomenon and cannot be established by histologic methods. Nevertheless, the direction of eruption bears a relation to the pattern of resorption.

The presence of deciduous tooth fragments in eccentric eruption had not previously been reported. These fragments are not similar to the root fragments described by Aisenberg.[1] It is evident that fragments can be produced by bizarre resorption patterns. When such patterns produce thin plates of root surfaces, trauma may fracture and displace pieces of the plate (Fig. 10). Whether the presence of fragments would deflect an erupting permanent tooth was not established by this investigation.

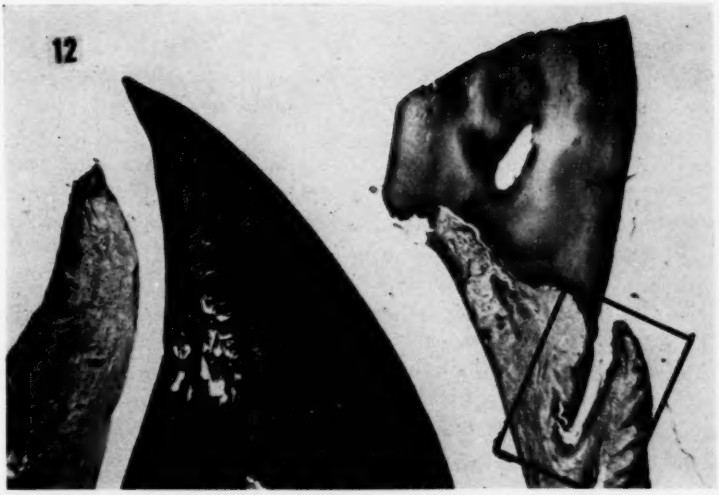

FIG. 12.—Lingual emergence of the permanent tooth. The deciduous tooth remains *in situ*. Note fusion of the oral mucosa with the odontogenic epithelium on the lingual.

FIG. 13.—High magnification of box in Fig. 12. Note odontogenic epithelium (*E*) of sulcus of the deciduous tooth migrating along the resorbed inner surface of the dentin.

It is likely that the disposition of these fragments may occur in two ways. If they are small enough, they may be completely resorbed. However, when epithelialization of the fragment occurs, resorption ceases. One may project, then, that the fragments are exfoliated when the oral mucosa and the odontogenic epithelium join to permit emergence of the permanent tooth.

There was an abundance of inflammatory cells in the gingival sulcus of the deciduous tooth at the time when shedding was imminent (Figs. 2, 11, 13). The epithelium was attracted to the dense inflammation. In the later stages it migrated toward the resorbing surface of the dentin, separating the connective tissue from the resorbed surface (Figs. 2, 11, 13). From histologic examination, the epithelium seemed to cover the connective tissue and not to be attached to the resorbing dentin. The osteoclasts were no longer present after the epithelium moved across the surface. Smith[2] showed that epithelial proliferation was directed toward the concentration of inflammatory cells in the isolation and exfoliation of root fragments in rats. A similar phenomenon occurred in the shedding of the deciduous tooth, as well as in the isolation of the tooth fragments in eccentric eruption.

The epithelial activity, after exfoliation of the deciduous tooth, explained the manner in which a keratinized layer was formed after the odontogenic epithelium first covered the wound. The odontogenic epithelium migrated, in part, across the surface prior to the exfoliation of the deciduous tooth. No single specimen obtained showed the extent of repair by odontogenic epithelium precisely at the moment of shedding. However, examination of Figures 3, 4, and 5 indicates the sequence of events. Immediately after shedding, the area of the wound was covered by odontogenic epithelium. At the margins, the oral mucosa moved over the surface to cover the odontogenic epithelium, as in Figure 4.

The ultimate disposition of the odontogenic epithelium in the repair of the wound caused by shedding was not observed in the static tissue sections. One can only speculate on degeneration, phagocytosis, or lysis of the odontogenic epithelium, since Figure 5 shows no evidence of its presence.

A dense layer of connective tissue between the erupting tooth and the oral epithelium was present in many sections (Figs. 3, 5, 8, 9). This was also observed in two of Aisenberg's illustrations.[1] By its location, it seemed to separate the dental sac from the connective tissue underlying the deciduous tooth. However, when the stratum intermedium of the permanent tooth enamel organ encapsulated the remaining fragments, the layer was not present.

A significant feature of the study was that the sulcus epithelium of the deciduous tooth made the initial repair of the wound caused by shedding of the tooth. It did not enter into the formation of the sulcus of the permanent tooth.

SUMMARY

The pattern of resorption of the deciduous tooth conformed to the position of the erupting permanent tooth. The permanent tooth might erupt directly beneath the deciduous tooth, or it might be labially or lingually inclined. In the latter instance, root fragments were found in the underlying tissue after exfoliation of the deciduous tooth. The odontogenic epithelium of the permanent tooth enveloped the root fragments when present.

The sulcus epithelium of the deciduous tooth participated in the repair of the wound and was ultimately replaced with masticatory mucosa. The sulcus epithelium of the deciduous tooth did not enter into the formation of the sulcus of the permanent tooth.

The author wishes to express his deep appreciation and indebtedness to Dr. William Lefkowitz, professor of dentistry, University of Kansas City, for his untiring efforts and guidance throughout the course of this investigation.

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The Relative Form of the Palate in Class I and Class II Malocclusions

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The form of the dental arches in man is described, to a considerable extent, by the relationship of one arch to the other. The directions in which this form is altered by an improper arch relation are defined in Angle's methods of classifying malocclusions.

Angle[1] separated malocclusion into three main categories based on the anterior-posterior relationship of the mandibular first permanent molars to the maxillary first permanent molars. Angle maintained that the Class I malocclusion was "characterized by normal mesial-distal relations of the jaws and dental arches, as indicated by the normal locking in eruption of the first permanent molars."[2] In Class II malocclusions, abnormal mesial-distal relations of the jaws and dental arches were said to occur, and certain variations from normal in the form of the arches could be noted. Angle described the dental arch form in Class II, Division I, malocclusion in this manner: "The upper arch is always abnormally lengthened and narrowed."[3] In Class II, Division II, malocclusion he said: "The width of the arches is more nearly normal."[4]

Since the question of palatal size associated with either Class I or Class II malocclusion has not been the primary objective of any previous research projects,[5-8] the present study was undertaken. This study is a direct analysis of the height, width, and length of the hard palate as seen in Class I and in Class II malocclusions, utilizing the data collected on 200 male subjects in the age range of thirteen through sixteen years. From these data, the relative areas of palate cross-sections (width $\times$ height, height $\times$ length, and length $\times$ width) of Class I and Class II malocclusions were statistically analyzed.

MATERIALS AND METHODS

Data for palate height, width, and length were obtained from 200 white male subjects in the age range of thirteen through sixteen years. These subjects were selected as to malocclusion: 100 with Class I malocclusion and 100 with Class II malocclusion (50 Class II, Division I; 50 Class II, Division II). A preliminary clinical examination of each subject was conducted to determine whether each subject fulfilled the following qualifications: (1) that he was not at present under, or previously had had, orthodontic treatment; (2) that the class of malocclusion was determinable and bilateral; (3) that the maxillary and mandibular first and second premolars were pres-

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ent and in occlusion; and (4) that the subject had no extracted or missing permanent teeth.

As each subject was presented, the class of malocclusion was recorded. The fovea palatina was then located and marked clearly with an indelible pencil. A maxillary dental impression was taken with fast-setting alginate impression material. The indelible pencil mark transferred to the impression in the process, and a straight pin was inserted into the impression material at this mark to locate more definitely the fovea palatina on the cast. The impression was poured in plaster, with the straight pin now imbedded in the plaster cast.

Three palatal dimensions were determined on each cast: (1) palatal height—from a plane connecting the gingival margin of the maxillary right and left first molars to the highest point of the palate in the mid-line; (2) palatal width—the minimum linear

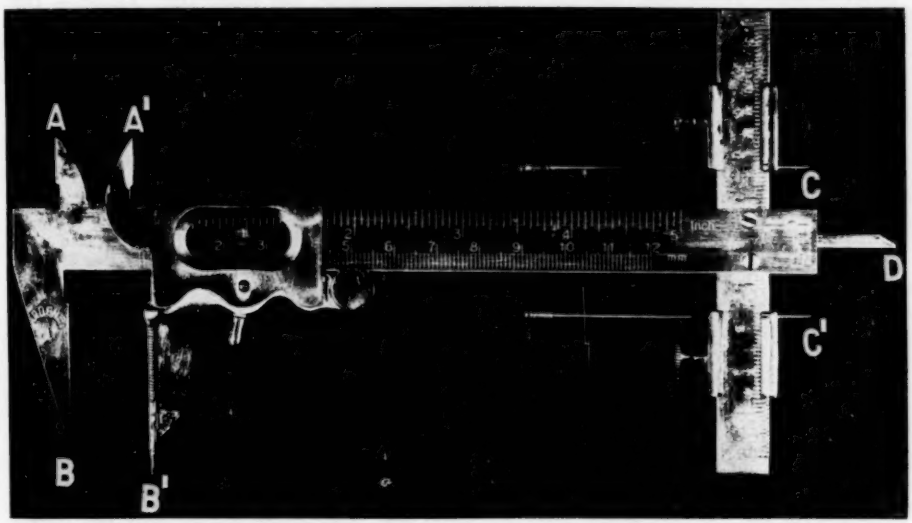

FIG. 1.—Photograph of measuring device. See text for a description of the use of this instrument

distance between the lingual gingival margins of the right and left maxillary first molars; and (3) palatal length—from a mid-point at the lingual gingival margin of the central incisors posteriorly to the fovea palatina.

All the casts were trimmed in such a manner that the backing side (side directly opposite the occlusal surfaces of the teeth) was parallel with the occlusal plane. The occlusal plane was established by a flat plane connecting the points of the cusps on the right and left first and second bicuspids and the right and left first permanent molars. This plane was used as the base plane for the directed measurements of palate height and length.

Measurements* were made to the nearest 0.1 mm., and only one dimension in turn was determined on the entire series of dental casts. As each measurement was obtained, it was recorded and grouped as to class of malocclusion. The measurement procedure was as follows (Fig. 1). The width measurements were first obtained with

* All measurements were made with a modified Helois Sliding Caliper (Fig. 1).

the outside calipers A and A' at the terminal points, locating the minimum linear distance between the lingual gingival margins of the right and left maxillary first molars. The length measurements were then obtained with the inside calipers B and B'. The main bar was held parallel to the occlusal plane by one end of a straight rod inserted into a lock clamp attached to the back of the main bar.

The opposite end of the straight bar was inserted into a lock-clamp attached on the vertical portion of an inverted L-stand. In this manner, the main bar was fixed in a parallel relationship to the established occlusal plane of each individual cast throughout the entire measurement procedure for palate length. Point B was placed on the anterior terminal point, and point B' was moved posteriorly to the area of the posterior terminal point. Definitely to locate the posterior terminal point, point B' was designed as a spring-loaded plunger mechanism and could be moved to the exact point without disrupting the fixed parallel relation of the main bar. Through this measurement procedure, a parallel straight-plane measurement for length was obtained. The third and final measurement, palate height, was obtained in the following manner: the end of the straight bar which had been inserted into the lock-clamp on

TABLE 1

MEAN PALATAL DIMENSION BY CLASS OF MALOCCLUSION

Class of Malocclusion	No. of Cases	Width* (Mm.) Mean S.D.	Height (Mm.) Mean S.D.	Length (Mm.) Mean S.D.
Class I	100	34.4±2.6	13.6±2.4	46.4±3.3
Class II (as a whole)	100	31.3±2.1	12.5±2.6	46.0±3.2
Class II, Div. I	50	31.1±2.4	12.4±2.5	46.5±3.3
Class II, Div. II	50	31.5±1.7	12.6±2.9	45.3±3.2

* For width, Class I versus Class II, $P = 0.001$. All other comparisons not statistically significant.

the vertical length of the L-stand was removed and inserted into a lock-clamp on the horizontal length of the L-stand. In this way the accessory bar was placed parallel and adjacent to the occlusal plane. The accessory bar was calibrated so that the palate height measurement could be taken in the exact mid-line. The mid-line was determined by calculating the mid-portion of the palate from the previous width findings of the particular cast. The points C and C' were then moved laterally to the desired points on the accessory bar, thus placing the height bar D in the mid-line. Points C and C' were then positioned at the same points as those from which the width measurements had been determined and the height bar D was extended to the height of the vault.

RESULTS

The greatest difference between palates in Class I malocclusion and Class II malocclusion was found in the means of width measurements (Table 1). This difference, Class I palates 2.8 mm. wider than Class II, was statistically significant ($P = 0.001$). The difference between the divisions of the Class II group and the differences between Class I and Class II as a whole were not statistically significant for any of the other comparisons.

To compare the palatal shapes seen in Class I malocclusion, Class II malocclusion,

and each of the subdivisions, cross-sectional areas were examined. Although the products of width × height, height × length, and length × width are not actual areas, they should give comparative values related to the actual areas. The three cross-sectional products were determined for each subject. From these data the mean and standard deviations were calculated for each malocclusion group—Class I malocclusion, Class II malocclusion (as a whole), and subdivisions I and II (Table 2). There was no significant difference between subdivisions I and II of Class II malocclusions. The mean values for both width × height and width × length were significantly greater ($P = 0.001$) in Class I malocclusion than in Class II (as a whole) malocclusion. The product of height × length showed no significant difference between Class I malocclusion and Class II malocclusion (as a whole) or the subdivisions.

TABLE 2

MEAN PALATE CROSS-SECTIONAL AREAS BY CLASS OF MALOCCLUSION

Class of Malocclusion	No. of Cases	Length × Width Cross-sectional Area (Square Mm.)		Width × Height Cross-sectional Area (Square Mm.)		Height × Length Cross-sectional Area (Square Mm.)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Class I	100	1599.24	±160.75	479.91	±69.60	629.00	±110.62
Class II as a whole	100	1437.22	±155.45	393.38	±80.08	580.31	±115.65
Class II, Div. I	50	1448.71	±157.49	388.12	±67.56	589.08	±110.78
Class II, Div. II	50	1429.10	±154.50	398.80	±91.27	571.54	±120.65

The values listed in Table 2 indicate that the palate cross-sectional products of height × length did not differ from one group to another, regardless of class of malocclusion. The mean values of the cross-sectional areas of height-width and length-width definitely showed a significant difference between Class I and Class II malocclusion (as a whole), pointing out that a definite change in the cross-sectional areas of length-width and width-height were associated with an abnormal arch relation seen in the Class II malocclusion.

DISCUSSION

The past literature has presented numerous biometrical studies in which measurements of palatal height, width, and length were integral parts of the investigative efforts. An analysis of those results reveals an apparent lack of agreement in many of the measurements. The conflicting results are due principally to the utilization of differing reference points or landmarks for measurements. Since this is a study in absolute size differences, only those studies—Talbot,[5] Alkan,[9] Channing and Wissler,[10] Hellman,[11] Franke,[12] Rittershofer,[13] Smyth and Young,[14] Brawley and Sedwick,[15] and Meredith and Cox[16]—in which height, width, and length measurements were taken from comparable terminal points will be included (see Table 3).

The established occlusal plane was not held as a reference plane from one subject to another but, rather, was used as a base plane to which all measurements of palate height and length in this study were directed. Since this occlusal plane was not a

reference plane common from one subject to another, certain errors were incorporated in the findings of this study compared with those findings that would have been found, had these measurements been referred to an accepted reference plane, such as the Frankfort Horizontal Plane. It was therefore necessary to relate the occlusal plane to the Frankfort Horizontal Plane, in order to calculate the possible percentage errors in the data of this study.

The angle variation of the occlusal plane with the accepted reference plane, the Frankfort Horizontal Plane, was determined from an analysis of cephalometric head plates. From these data were calculated the percentage error in the measurement procedure, had these measurements been directed to the Frankfort Horizontal Plane rather than the established occlusal plane.

TABLE 3

MEAN PALATE MEASUREMENTS LISTED FOR FORMER STUDIES AND PRESENT STUDY

AUTHOR	YEAR	CASES	RESULTS		
			Width (Mm.)	Height (Mm.)	Length (Mm.)
Talbot (5)	1892	4,614	.....	14.7	.....
Alkan (9)	1900	5	.....	15.0	.....
Channing and Wissler (10)	1908	165	33.9	13.8	.....
Franke* (12, 14)	1921	57	33.5	.....	.....
Hellman (11)	1927	10	.....	11.35	43.3
Rittershofer (13)	1931	12	.....	8.9	.....
Smyth and Young (14)	1932	57	35.6	.....	.....
Brawley and Sedwick (15)	1939	189	35.4	14.6	38.1
Meredith and Cox† (16)	1954	44	34.4	.....	.....
Staab	1960 Class I	100	34.1	13.6	46.4
	Class II (as a whole)	100	31.3	12.5	46.0

* Franke's data as recalculated by Smyth and Young.

† Authors made two measurements to determine arch width, on the lingual and on the buccal surfaces of the first permanent molars. Only the lingual measurements are included in this table.

In an examination of 75 head plates of other subjects, 40 of which were Class I malocclusion types and 35 of which were Class II malocclusion types, it was found that the angle that the occlusal plane formed with the Frankfort Horizontal Plane ranged from 7° to 20°, with the average being 13°. The findings of percentage difference from the values, had the original measurements of palate height and width been directed to the Frankfort Horizontal Plane, were calculated for the greatest, the least, and the mean angle variations with the greatest, the least, and the mean height variations and the greatest, the least, and the mean length variations of the subjects studied. These calculations indicated that the maximum error that could be expected because of using the occlusal plane would be less than the experimental error of the measuring instrument.

The results listed in Table 1 are in accord with Angle's observations of the form of the dental arch in Class II, Division I, malocclusions, as "abnormally narrowed," but do not support his observation of "more nearly normal" for Class II, Division II.

The three cross-sectional products used in this study were selected because, through these selected areas, the concept of palate shape was separated into three distinct aspects. In this way the shape of the palate could be described and analyzed by three numbers.

The shape of the palate, as considered in this study, depends on three basic dimensions: palate height, palate width, and palate length. It can be seen that a change in any one of the basic palate dimensions brings about a change in two of the three cross-sectional products. Thus, as was shown in this study, Class I and Class II malocclusion palates differ only in the basic dimension of width. However, this one dimensional difference, when considered as the change it produces on shape, becomes a major factor in describing the palate shape in Class I and Class II malocclusions.

SUMMARY

This study was designed to examine the relationship between certain palate dimensions and the malocclusion according to Angle's classification. Measurements of width, height, and length were made on maxillary study casts taken from 100 white males having Class I malocclusion and 100 white males having Class II malocclusion (50 cases each Division I and Division II). The mean width was less in Class II than in Class I, with no significant differences between lengths or between heights. There were no differences between Class II, Division I, and Class II, Division II.

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The Formation of Collagen Fibrils in the Dental Pulp

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In the last twenty years, because of the application of the electron microscope, there has been a considerable increase in knowledge concerning the origin and mechanism of formation of collagen. Since the work of Schmitt, Hall, and Jakus[1] in 1942 illustrated that collagen was composed of a unit structure of fibrils, renewed interest has appeared in this area. Porter[2] in 1952 and Wasserman[3] in 1956 have reviewed the extensive work in this area and pointed out that the two controversial viewpoints of intracellular versus intercellular origin of collagen still persist. Fleming,[4] Mall,[5] Ferguson,[6] and Lewis[7] promoted the theory that collagen was formed within the cell. These beliefs were later supported by Jackson,[8] Jackson and Smith,[9] and Wasserman[3, 10] by the observation of submicroscopic filaments or primary fibrils within the cytoplasm. On the other hand, Baitsell,[11-13] Baitsell and Mason,[14] Maximow,[15] Nageotte and Guyon,[16] Levi-Montalcini and Sacerdote,[17] and more recently, Stearns[18, 19] and Kajikawa and Sumita[20] point out that collagen is formed outside the cell in the ground substance. Studnicka,[21] Porter,[2] and Porter and Pappas[22] have arrived at perhaps a third viewpoint in their belief that the collagen fibril components are developed extracellularly but at, or on the surface of, the fibroblast. Jackson[23] and Jackson and Smith[24] now support the theory that fibrogenesis is intimately associated with the surface of osteoblasts and chondroblasts, as does Wasserman,[10] who observed bundles of primary fibrils at the surface of fibroblasts. Bradbury and Meek[25] have recently illustrated collagen fibrils in the covering on the surface of fibroblasts in the leech. Weiss and Ferris[26] and Kemp[27, 28] came to a similar conclusion that procollagen units are derived from the fibroblasts. The majority of the recent evidence supports the premise that collagen is formed near, or in close conjunction with, the cell surface and that precursor substances are formed within the cell.

The tooth pulp has been described as being composed of a specialized connective tissue syncytium of stellate-appearing fibroblasts.[29, 30] There have been few electron-microscope studies of the pulp except those describing the fine structure of nerve fibers by Arwill,[31] Matthews, Dorman, and Bishop,[32] Uchizomo and Homma,[33] and Engstrom and Ohman.[34] To the authors' knowledge there have been no descriptions of where the collagen fibrils of the pulp arise. The present study was designed to obtain information about the morphologic characteristics of the pulp fibroblast and to gain some insight into the origin of the collagen fibrils of the pulp.

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MATERIALS AND METHODS

Eighty young molar tooth buds were removed from newborn hamsters and fixed for 1½ hours in either 2 per cent osmium tetroxide buffered at pH 7.2 with veronal acetate or in 2.4 per cent potassium permanganate[35] with 0.14 M veronal acetate buffer and calcium chloride added to make a 0.1 per cent solution. The molar tooth buds, measuring less than 1–2 mm. in diameter, were fixed without trimming and then dehydrated in 30, 50, 70, 80, and 95 per cent absolute ethyl alcohol for a total time of 1 hour and 30 minutes. The tissues were infiltrated in equal parts of absolute alcohol and butyl methacrylate for 30 minutes and then in 100 per cent butyl methacrylate for 30 minutes. The tissues were then placed in an 80 per cent butyl methacrylate and 20 per cent methyl methacrylate mixture for 30 minutes, after which time the catalyst* was added, and the tissues were allowed to stand 30 minutes. Then they were transferred to gelatin capsules containing catalyzed 80:20 butyl:methyl methacrylate mixture and placed for 30 minutes in a vacuum chamber at 15 inches Hg. The capsules were then placed in a 50° C. oven for curing. After 24 hours the plastic-imbedded tissues were sectioned on a microtome† at approximately 25–90 millimicrons.

RESULTS

The potassium permanganate appeared to provide better fixation of cell membranes, certain intracellular structure, and ground substance of young pulp than did the osmium tetroxide in the manner used. Apparently, the gelatinous young pulp, surrounded by dentin and enamel matrix, was penetrated less effectively by the OsO_4 than by the $KMnO_4$. The observations of the present study therefore will be confined to the permanganate-fixed material.

Fibroblasts.—In young pulp tissue the cells found in greatest numbers were the fibroblasts or fibrocytes. These cells appeared to vary in shape, most of them having a stellate appearance with from one to several cell processes seen in any plane (Figs. 1–4). The width of the cell body ranged from 9 to 15 μ. The cell processes were found to reach 22 μ in length or even longer. The fibroblasts did not form a true syncytium with adjacent cells. The cell membranes were observed to inclose the cytoplasm completely, and desmosome-like structures were noted between cell processes and probably functioned in attachment between cells (Figs. 3 and 13). Most cells had round or oval-shaped nuclei with two or three small, indistinct nucleoli (Figs. 3 and 4). The nucleoplasm and the nucleoli appeared less electron-dense with $KMnO_4$ than with OsO_4. The nuclear membrane was found to be double (Figs. 4 and 10), exhibiting the characteristic pores (Fig. 4). The cytoplasm of the cells appeared densely populated with oval mitochondria and, to a lesser extent, with endoplasmic reticulum (Figs. 3–5). A few mitochondria varied considerably from the oval shape, ranging in length from 0.5 to 6 μ (Fig. 7), but the average size was found to be 0.75 μ in length. The mitochondria did not appear to have regularly spaced cristae mitochondriales extending across the structure. The double external membranes of the mitochondria and the cristae mitochondriales were clear, the membranes being thinner than the cristae (Figs. 7 and 12). The endoplasmic reticulum was not highly organized and was scattered in tubular

* Luperco CDB, Wallace and Tiernan, Inc., 1740 Military Road, Buffalo, N.Y.

† Porter-Blum.

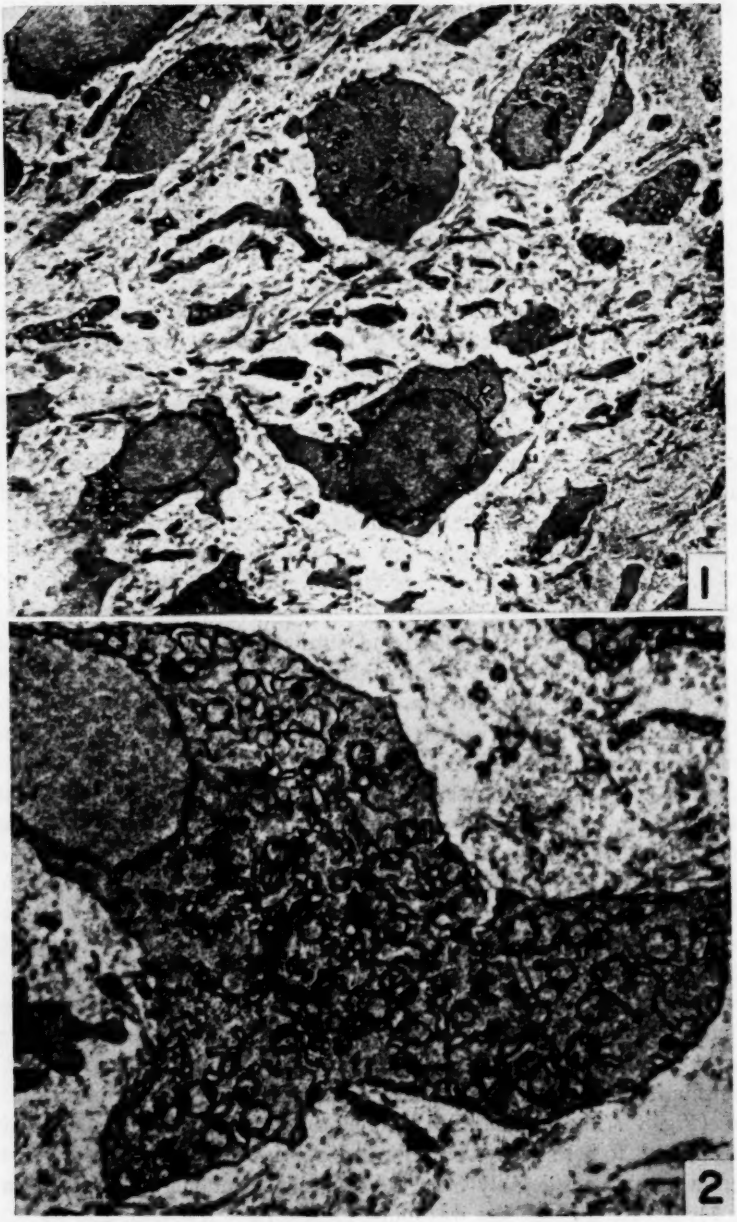


FIG. 1.—The central zone of young pulp, illustrating numerous fibroblasts and collagen fibrils scattered throughout the area. (Total mag. ×2,000.)

FIG. 2.—A typical fibroblast illustrating the oval, lightly stained nucleus. Numerous mitochondria are seen scattered throughout the cytoplasm. The Golgi apparatus is located near the nucleus. Numerous collagen fibrils are visible near the cell processes. (Total mag. ×4,693.)

FIG. 3.—A fibroblast of the young pulp with cell processes extending above and to the right. A small nucleolus is seen in the left part of the nucleus. Absence of, or decrease in number of, cristae mitochondriales in many of the mitochondria may indicate that they are immature. (Orig. mag. ×5,280.) The insert in the upper right corner illustrates an intercellular attachment between two fibroblasts. The nuclei of the two cells are seen in the upper right and lower left. (Total mag. ×5,675.)

FIG. 4.—A higher magnification of a fibroblast with an oval nucleus. An island of collagen fibrils surrounded by a membrane appears near the point of the arrow (*lower left*). This inclusion is probably due to an infolding of the outer cell membrane along with the adjacent fibrils. (Total mag. ×7,548.)

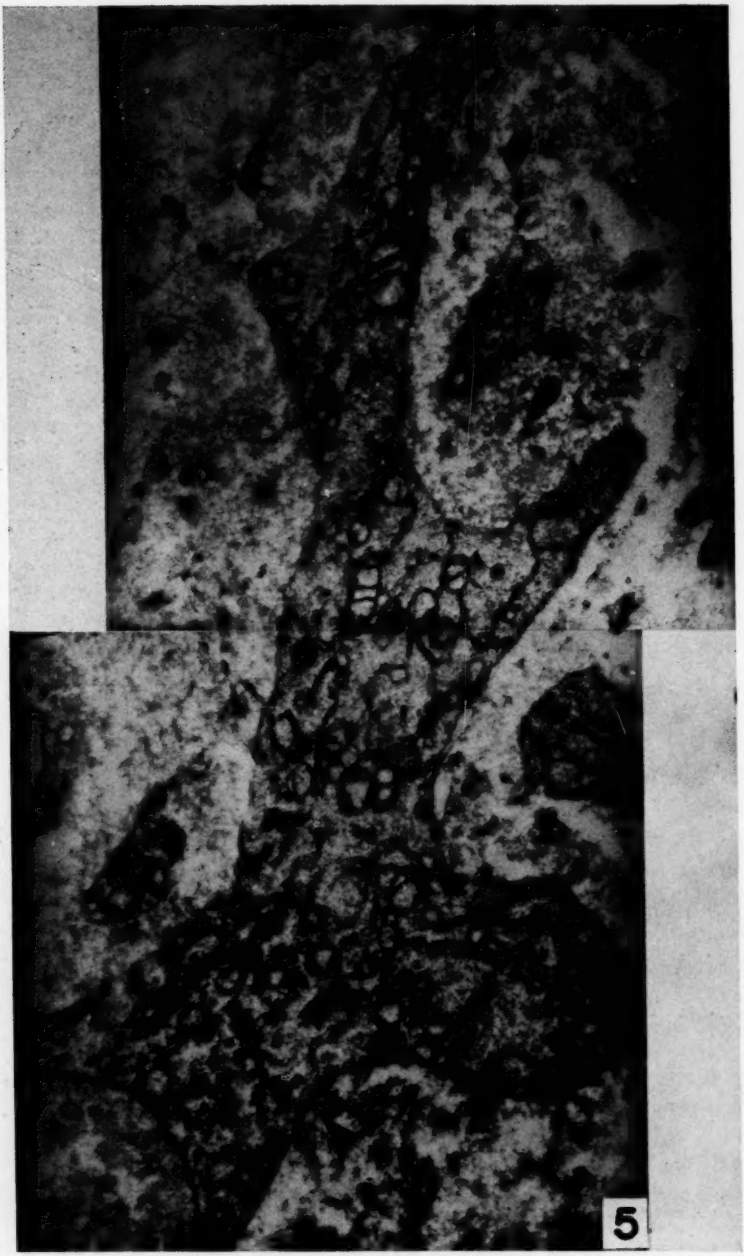


FIG. 5.—A composite electronmicrograph of a pulpal fibroblast and three of its processes. Part of the nucleus is shown (*lower left*). The Golgi apparatus is visible near the nucleus. Endoplasmic reticulum and numerous mitochondria are scattered throughout the cytoplasm. The arrow points to collagen fibrils located near the surface of the cell. (Total mag. ×6,875.)

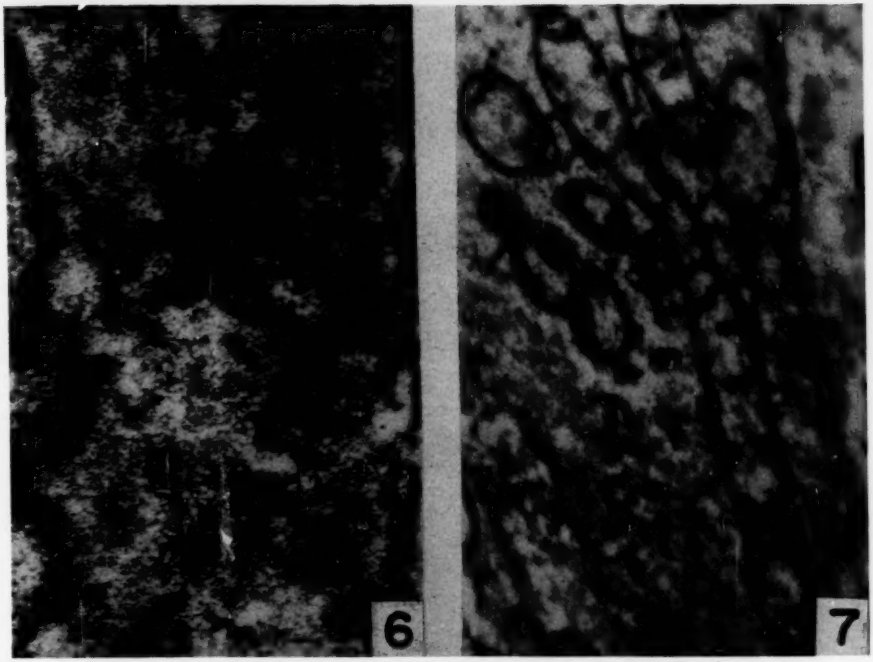


FIG. 6.—A high-magnification electronmicrograph of a typical Golgi apparatus in a pulp fibroblast. It appears as a group of bilaminar structures with closed ends and several vesicles. (Total mag. ×43,200.)

FIG. 7.—A high-magnification electronmicrograph of typical mitochondria of pulp fibroblasts illustrating the great variations in length of these structures. The mitochondrion on the right measures 6.7 μ. The cristae mitochondriales are few in number and randomly spaced. (Total mag. ×14,900.)

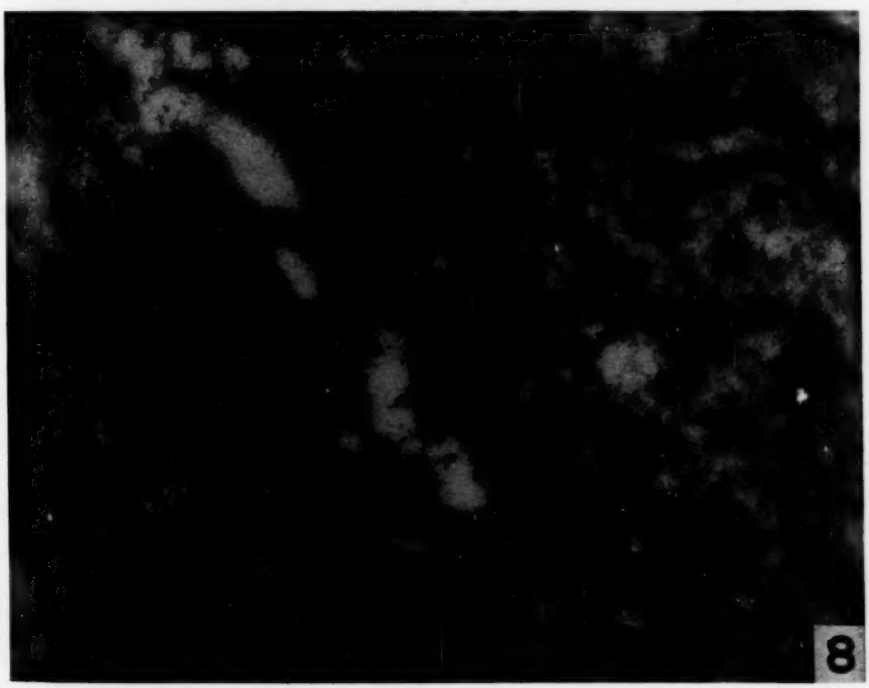


FIG. 8.—A high-magnification electronmicrograph of collagen fibrils near the outer cell membrane of the fibroblast. The collagen periodicity is noticeable. (Total mag. ×46,080.)

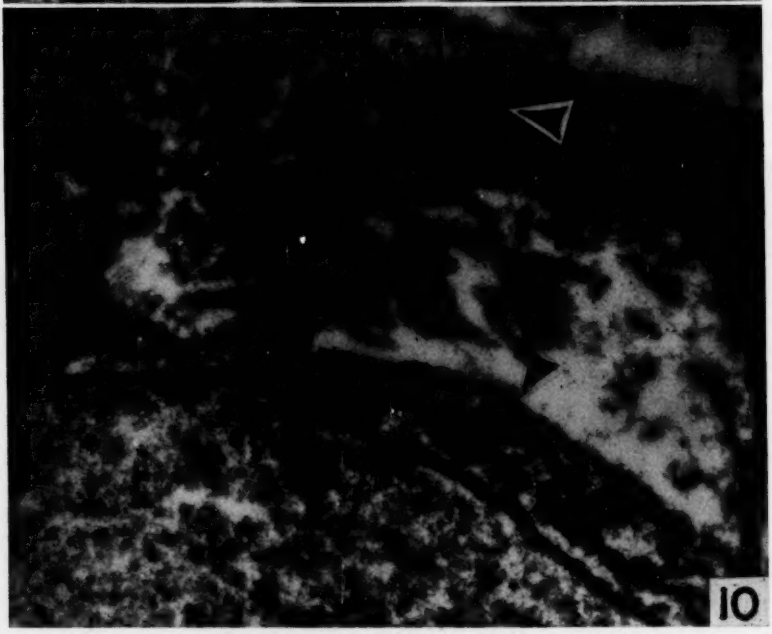
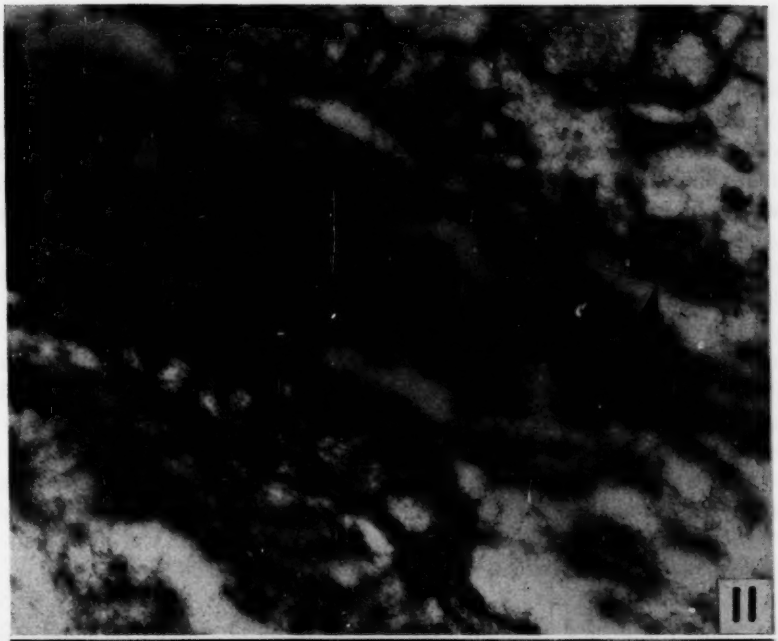


FIG. 9.—Numerous collagen fibrils lying near the outer cell membrane of a fibroblast process in the pulp. The single layer of the outer cell membrane is visible. (Total mag. ×23,936.)

FIG. 10.—Young collagen fibrils lying near the surface of a pulp fibroblast. Near the point of the upper arrow some fibrils appear to be located within the cytoplasm of the cell. This appearance is believed due to the presence of the fibrils lying adjacent to the outer cell membrane, which lies either immediately above or below this section. In the lower part of the picture is seen the double membrane of the nucleus with a narrow zone of cytoplasm just above it. The outer cell membrane is indistinct, and a collagen fibril (*lower arrow*) lies in close approximation with the cell surface. (Total mag. ×17,770.)



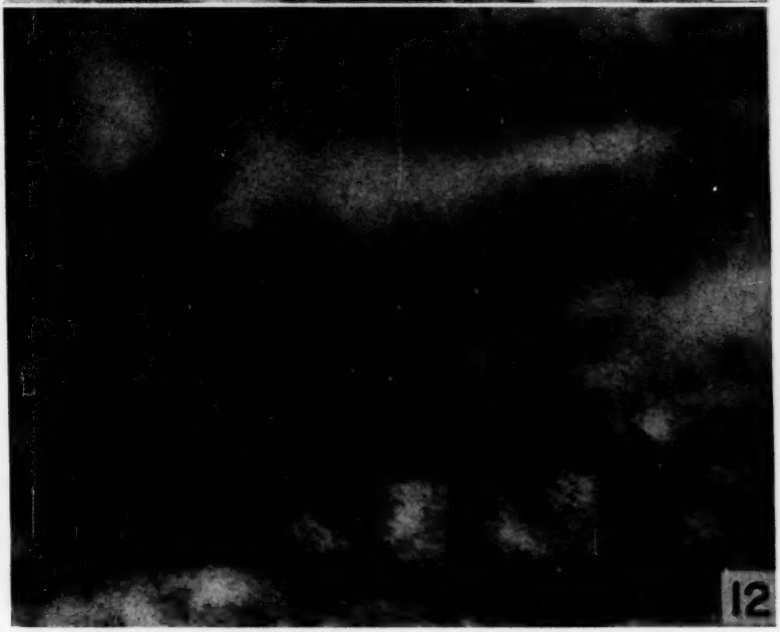


FIG. 11.—Young collagen fibrils lying in contact with the outer cell membranes of pulp fibroblasts. Near the points of the arrows the collagen fibrils appear to be in contact with, or imbedded in, the cell surface. (Total mag. ×26,293.)

FIG. 12.—A higher magnification of the lower half of Fig. 11. The double wall of the mitochondrion is seen in near contact with the single-layer cell membrane. The outer part of the cell wall appears "ruffl l" or altered, and at the point of the arrow is seen one end of a collagen fibril, which appears to be imbedded in, or lying in contact with, the cell wall to the left. (Total mag. ×68,000.)

form throughout the cell (Fig. 5). With $KMnO_4$ fixation the ribosome granules were not preserved and thus not apparent on these structures (Fig. 8). The Golgi apparatus was located near the nuclei of these cells and appeared as clusters of short, bilaminar membranes with several associated vesicles (Figs. 2, 5, and 6). In some instances the bilaminar components appeared straight, while in other cells they formed semilunar-shaped structures. The cytoplasm appeared granular, with no increase in density seen in any particular area of the cell or its processes. A number of small rodlets, particulate matter, and other electron-dense structures appeared scattered throughout the cytoplasm of the cell.

The fibrils of the intercellular substance.—In the area immediately surrounding the cell, numerous bundles of fibrils were scattered in the ground substance (Figs. 1–3 and 5). Examination at higher magnifications revealed that these fibrils had the banding characteristic of collagen. It was observed that many of these fibrils lay in close association with the cell and, in some instances, in contact with the outer cell membrane (Figs. 8–12). At times the fibrils were lying at right angles to the cell surface but usually were found in a plane similar to that of the outer surface of the cell.

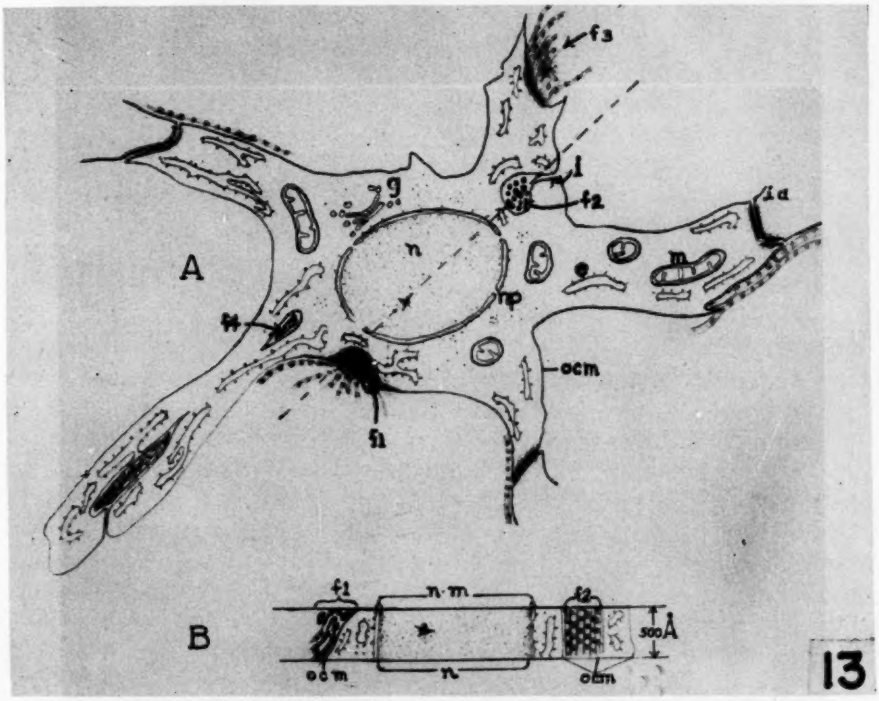


FIG. 13.—A diagrammatic representation of the electron-microscopic appearance of a young pulpal fibroblast as visualized in the present study. *A:* Top view of a sectioned fibroblast. *B:* A cross-section view of the 500 A-thick section *A* along dotted line. *n*, nucleus; *nm*, nuclear membrane; *np*, nuclear pore; *ocm*, outer cell membrane; *g*, Golgi apparatus; *m*, mitochondria; *e*, endoplasmic reticulum; *i*, infolding membrane; *ia*, intercellular attachment. Fibrils (*f1* and *f3*) superimposed on the sloped portions of the outer cell membrane will cause the cell membrane to be completly obscured, producing the appearance of cytoplasmic continuity with the fibrils. At area *f2* the fibrils appear to be completely surrounded by cytoplasm, as in *f4*.

Although the majority of the fibrils were closely associated with the cell processes, some were found adjacent to the cell body as well. In some electronmicrographs the fibrils appeared to have an intracellular location. The observation was believed to be an artifact because of the following reasons. In some cases the intracellular fibrils appear located in a space which is completely surrounded by a membrane which appears structurally very much like the outer cell membrane (Fig. 4). Since the outer cell membrane follows a tortuous course, it is believed possible that an infolding of the cell surface and adjacent fibrils could be included in any section through the cell. Other groups of fibrils appeared to be located within the cytoplasm near the cell surface. In these cases no outer membrane confining the fibrils was observed within the cell (Fig. 10). In such instances it was believed that the section was made almost parallel to the surface of a fibril-covered outer membrane, which then slanted upward out of the plane of section. However, the membrane is so thin and in such close contact with the fibrils that it cannot be distinguished from the cut ends of the fibrils, causing them to appear to be in contact with the cytoplasm. A diagrammatic explanation of the authors' interpretation of the finding of apparently intracellular collagen fibrils is shown in Figure 13. In a few cases collagen fibrils with a diameter of 100–300 A were observed imbedded in, or in close association with, the outer membrane of the cell (Figs. 10–12). In Figures 11 and 12 it can be seen that some alteration has possibly occurred in the cell membrane in the area where a fibril is located. Although the cell membrane is intact in these areas, the outer portion of it appears altered in respect to the adjacent fibril. Measurements were made of the diameter and periodicity of the numerous collagen fibrils throughout the pulp. At a distance from the cell surface the fibrils were found to measure approximately 200–400 A in diameter and to exhibit 458 A periodicity. It was not possible to obtain accurate measurements of the length of the fibrils because an entire fibril was probably not included in the plane of a single thin section.

DISCUSSION

The young fibroblasts found in the hamster dental pulp appear somewhat less differentiated than fibroblasts found in the adult rat.[36, 37] The nucleoli of the young pulp fibroblasts are smaller, the mitochondria are less differentiated, and the endoplasmic reticulum is more sparse or less highly developed. In the adult fibroblasts the perinuclear cytoplasm is more dense than that found in their processes, whereas the density of the cytoplasm in the young pulpal fibroblasts does not vary throughout the cell and its processes. These variations in characteristics may indicate that the cells found in the young pulp are not fully differentiated. On the other hand, these cells appear differentiated sufficiently to function in fibril production. Perhaps they will develop into more mature cellular elements of the pulp which function in the formation of collagen fibrils important to dentinogenesis. Further studies on these cells is necessary before such conclusions can be made, however.

The present study indicated that collagen fibrils of the dental pulp were formed at the surface of the pulp fibroblast. These young fibrils measured only 250 A in diameter, as compared to those measuring 600 A found in hamster dentin by Watson and Avery.[38] On the other hand, those authors found collagen fibrils measuring 100–300 A in diameter along the dentinal tubule in predentin, which indicated that some

fibrils were first deposited in the peritubular region and continued with age to increase in diameter. Porter and Pappas[22] noted fibrils in the region of the cell surface of chick fibroblasts to be 200–300 A in diameter. It is their belief that fibrils smaller than 600 A grow by addition to their surface of monomeric collagen and not by association with groups of fibrils. It is not understood why the short periodic lengths of 458 A were found in the present study. It is possible that the 640 A period characteristic of collagen was not fully established in these fibrils and that further organization of these fibrils occurred until the 640 A banding was attained.

Little is known concerning the mechanism of formation of collagen precursors by the fibroblasts. Jackson[8] and Jackson and Smith[9] showed intracytoplasmic rodlets which they believed to be precursors of collagen. Perhaps if Palade's[39, 40] theory of continuity of the endoplasmic reticulum with the cell membrane is correct, the possibility of intracisternal proteinic materials directly secreted into the intercellular spaces can be readily explained. However, there are claims by Karrer[41] that the unit structure of the cell membrane is different from that of the endoplasmic reticulum. Gross, Highberger, and Schmitt[42] suggest that collagen in a monomeric form is developed within the cell and is transformed into fibrils extracellularly. Porter[2] and Porter and Pappas[22] believe that the cell surface functions in some way that aids in the formation of the collagen template. Possibly at this site, polymerization of the forming substances may occur.

The findings in the present study can be interpreted in several ways in respect to fibrogenesis. Intracytoplasmic fibrils were noted, which could indicate intracellular formation, but these were believed to be present as a result of infolding of the outer cell membrane, since cell membranes were clearly visible surrounding groups of these fibrils. Other fibrils within the cytoplasm did not appear to be surrounded by membranes. In a 500 A-thick section, such as the ones in this study, it was believed possible for the outer membrane to be obscured by the cut ends of the fibrils as both slanted upward out of the plane of section.

It is apparent that the authors are avoiding the conclusion that unit fibrils are formed intracellularly. This is due partially to the fact that the apparently intracellular fibrils noted in the present investigation can be explained as being intercellular and also because the majority of findings by other investigators indicate that, although the procollagen molecules may be formed within the fibroblast, the unit fibril becomes recognizable extracellularly. In the case of the fibroblasts of the young pulp, the fibrils appear near or along or partly within the outer cell membranes. It is, therefore, possible that the outer membrane of the cell does play some part in the formation of these fibrils, as was believed by Porter and Pappas.[22]

It is indicated that further studies of the internal structure of fibroblasts and their membranes must be made before conclusions concerning the precise role of cytoplasm in collagen formation can be drawn. It is obvious from the present investigation that the dental pulp offers an excellent site for such studies. Although the young pulp fibroblasts studied appeared to be less differentiated than fibroblasts found in other areas of the adult animal body,[36] this was probably due to the embryonic nature of the young pulp. It will be necessary to study the fibroblasts at various ages of the pulp before their characteristics can be described fully.

SUMMARY AND CONCLUSIONS

Eighty molar buds from newborn hamsters were fixed in either $KMnO_4$ or OsO_4, imbedded in methacrylate, sectioned on a Porter-Blum microtome, and studied under the electron microscope. The $KMnO_4$ fixation was found to be far superior to OsO_4 for the study of the membranes of fibroblasts and fibril formation in young pulp.

The fibroblasts in young pulp were found to have an outer cell membrane completely inclosing the cytoplasm. Therefore, they do not form a true syncytium but establish contact with each other by means of intercellular attachments.

The cytoplasmic characteristics of fibroblasts in young pulp appeared somewhat different from those of fibroblasts found in connective tissue in adult animals. A lesser amount of endoplasmic reticulum was found, and the mitochondria in the young cells had fewer cristae mitochondriales. The electron density of the cytoplasm of the cell body was observed to be similar to that of its processes.

Intracellular location of collagen fibrils was observed but was believed to be artifactual and caused by sectioning through infolding membrane or oblique sectioning of the outer cell membrane. Many collagen fibrils measuring 100–300 A in diameter and having a periodicity of 458 A were observed near, or in contact with, the outer cell membrane of the fibroblast. Other collagen fibrils measuring 200–400 A in diameter were noted farther from the cell surface.

It is believed that the fibroblasts of the dental pulp function in the production of collagen fibrils on or within the outer membrane of the cell.

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Mandibular Fractures as Related to the Site of Trauma and the State of Dentition

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With sufficient energy imparted to the mandible by an impact, the bone will fracture. But where? Is the location of mandibular fractures haphazard and arbitrary, or are there predisposing factors which tend to influence this location? No doubt the shape of the bone itself plays an important role in determining the location of some of the fractures. The narrow neck of the mandible, with its reduced cross-sectional area, is probably less strong than the body or the symphysis. Also in this regard the magnitude of the energy of the impact will be significant. If the amount of energy imparted to the mandible is above the elastic limit of the bone, a fracture will occur, with most of the energy being dissipated in producing the one fracture. However, if the magnitude of the energy greatly exceeds the elastic limit, more fractures are likely to be produced.

Does the state of dentition or the site of trauma have any influence on the location of these fractures? Up to this time, no clinical or experimental evidence has been presented to substantiate or refute these questions. It is the purpose of this report to present data regarding the relationship of the location of mandibular fractures and number of fractures with the site of impact, the state of dentition, and the age of the individual.

MATERIALS AND METHODS

For this study, 319 case histories of mandibular fractures from The University Hospital, The University of Michigan, Ann Arbor, were analyzed. These case histories were also used in a previous study of mandibular fractures.[1]

In this study the data were placed in one of five categories based on the state of dentition of the mandible prior to trauma. The first three categories are edentulous, dentulous, and deciduous or mixed dentition. Patients with a number of teeth missing in a certain area of the mandible were placed in a fourth category called "quadrants missing"; these areas included the right and left posterior regions and, at times, the anterior region. The fifth category included all patients who had one or several scattered mandibular teeth missing; almost always these were molar teeth, usually the second and third molars. Each category was then subdivided, using a specific site of trauma for each subdivision. In all cases, information on the amount of energy of the impact imparted to the bone was not available.

The locations of the fractures were presented as subcondylar, at the neck of the

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mandible; angle-body, fractures located between the gonial angle and the mental foramen; chin fractures, between the mental foramina; and "other" fractures, including those of the ramus, alveolar bone alone, and the coronoid process.

The various sites of trauma used in this study were: chin point, the symphysis; lateral chin, between the symphyseal area and the corner of the mouth; chin area, the region between the corners of the mouth (this term was coined when some case histories stated the site of trauma as "to the chin" with no other information available; it refers to the anterior part of the jaw); angle or body, the side of the mandible; unknown, the site of trauma not known.

After the data were grouped into the various categories, they were subjected to statistical analysis by applying the chi-square test for significant association. Quite briefly, this method tests the hypothesis that two or more attributes are associated for causes other than a random (chance or accidental) combination. This test provides an excellent tool whereby objective statements can be made concerning non-metric (unmeasurable) data. The total number of cases that had a particular pair of attributes (for example, subcondylar fractures and dentulous jaws) was recorded in the proper square or cell (see Table 1), and these were termed the "observed frequencies."

TABLE 1*

ASSOCIATION BETWEEN STATE OF DENTITION AND LOCATION OF FRACTURE

State of Dentition	Fracture Location				Total
	Subcondylar	Angle or Body	Chin	Other	
Individual teeth missing	70.58 +0 / 79 (40)	80.86 / 72 (37)	27.41 + / 36 (18)	18.17 / 10 (5)	197
Dentulous	70.58 / 61 (31)	80.86 +0 / 85 (43)	27.41 / 25 (13)	18.17 + / 26 (13)	197
Edentulous	19.35 / 12 (22)	22.16 0 / 33 (61)	7.51 / 4 (7)	4.98 / 5 (9)	54
Quadrants missing	30.45 0 / 36 (42)	34.89 / 33 (39)	11.83 / 7 (8)	7.83 / 9 (11)	85
Deciduous and mixed	15.05 0 / 18 (42)	17.24 / 13 (30)	5.84 / 8 (19)	3.87 / 3 (9)	42
Total.....	206	236	80	53	575

* $N = 575$ (sample size) [319 cases]; D.F. = 12; $X^2 = 29.051$; $P = 32.909\ (0.001), 26.217\ (0.01), 24.054\ (0.02), 21.026\ (0.05)$. Within each cell, the value above the diagonal line is the computed expected frequency of the occurrence of the particular association. The value below the diagonal line is the observed or actual frequency of the particular association. The values in parentheses below the diagonal line within each cell represent the percentage of the particular observed frequency to all others in the table. The occurrence of a "+" above the diagonal line, below the expected value, indicates that the cell has the highest observed value for the particular column. A zero beneath the expected frequency indicates that the cell has the highest observed frequency for that particular row.

"Expected frequencies" were then computed. The expected frequencies represented the number of cases of the particular pair of attributes which would be expected to occur in the sample as a function of sampling error, that is, those cases in the sample which were in association purely by chance alone. Expected frequencies were calculated by multiplying the total number of one attribute—say subcondylar fractures (Table 1)—by the total number of another, dentulous jaws, and dividing by the grand total of all cases presented in the table ($206 \times 197 \div 575 = 70.58$). For each cell the difference between the observed and expected frequencies was squared, and this was divided by the expected frequncy. All these results were then added to obtain the chi-square value. The formula and a fuller explanation of the test can be found in any basic statistical text.

Once the chi-square value had been obtained, statistical tables were consulted, in order to determine the probability of obtaining the observed value if there were mere random (chance) association between the given attributes, taking into account the proper "degrees of freedom." The degrees of freedom were calculated by multiplying the number of rows in the table minus one, times the number of columns minus one. Using Table 1 as an example, there are four columns and five rows. The degrees of freedom then are $(4 - 1) \times (5 - 1) = 12$.

In this study, non-random associations of the attributes, that is, results not due to chance alone, were considered to be demonstrated only when the chi-square values exceded the 0.01 level of probability. That is to say, the results were due to chance and chance alone in one out of 100 samples. Probability values at the 0.001, 0.02, and 0.05 levels are included in each table to provide comparative information. In view of the high values of all the chi-square tests included herein, Yates's correction factor was not employed except to verify the assumption that its omission did not significantly alter the results.

Stated simply, the chi-square test allows the investigator to ask, "What is the probability that the association observed is due to chance alone?" Reference to Table 1 shows that the chi-square value was 29.051 when the state of dentition and the location of fractures were compared. The probability (P) at the 0.01 level was 26.217, indicating that these results would have been expected to occur only one time in 100 as a result of sampling error, that is, through chance alone in sampling. Since the obtained chi-square value (29.051) exceeded P at the 0.01 level (26.217) and, in fact, was approximately halfway between the values for the 0.01 and 0.001 levels, there was considerable justification for inferring that the state of dentition was significantly associated with the location of the mandibular fracture. The chi-square test eliminated subjective inference and objectively demonstrated that the association would be due to chance in less than one out of 100 randomly selected fracture cases.

The chi-square test is always set up on a "null" hypothesis. The null hypothesis is an assumption that the association in question is random and is not significant, being due only to chance. In Table 1 the hypothesis is that there is no association between the state of dentition and the location of fracture. The hypothesis, having been refuted (see above), allows for the statement that there is an association which is *not* due to chance alone. Therefore, "proof" lies in the refutation of the null hypothesis.

Although there is no adequate statistical method whereby the most significant cell associations can be determined within a given chi-square contingency table, it is pos-

sible to determine which *groups* of attributes are randomly or non-randomly associated within the given table. This was effected by applying additional chi-square tests to the data. Each table was examined to determine the location of the cell with the highest observed frequency within each column and within each row. These are indicated schematically within each of the chi-square tables; a plus sign designates the cell with the highest observed frequency of all the cells in a particular column, while a zero indicates the cell with the highest observed frequency of all the cells in a particular row.

Once this has been done, additional questions may be asked of the data through utilization of the chi-square test. As regards the pattern of the "+" signs (columns), the null hypothesis is that the association of these attributes to each other is not significant. The same hypothesis is made for the distribution of the highest observed frequencies by rows within the particular table. A third hypothesis is that the residual columns and rows are not significantly associated. Refutation of either or both of the first two null hypotheses indicates that, within the over-all table, certain groups of attributes are non-randomly associated. Acceptance of the null hypothesis when the chi-square test is applied to the residual cells indicates that they are associated only in a random manner.

This can be demonstrated by referring to Table 1. Here the distribution of the cells with the highest observed frequencies within each column is in the first two rows ("Individual Teeth Missing" and "Dentulous" jaws). The distribution of the cells with the highest observed frequencies for each row falls within the "Subcondylar" and "Angle-Body" fracture-site columns.

Applying the chi-square test to the first two rows, the chi-square value was 12.487, and, with three degrees of freedom, $P = 11.345$ (0.01) and 16.268 (0.001). Thus the association of dentulous jaws and jaws with individual teeth missing with the various fracture sites was considered to be non-random.

Applying another chi-square test to the cells which comprise the "Subcondylar" and "Angle or Body" fracture-site columns, chi-square equaled 13.020; with four degrees of freedom for this particular test, $P = 11.668$ (0.02) and 13.277 (0.01). The null hypothesis was not refuted, but the results were so close to the 0.01 confidence level that the non-randomness of the association was accepted with some reservations.

A final chi-square test was applied to the two residual columns and rows. Results indicated that the chi-square value was 2.519, and, at two degrees of freedom, $P = 2.408$ (0.30), 3.219 (0.20). Therefore, the null hypothesis was not refuted, and the association between these residual cells was quite random.

From these additional tests a further statement as to the association between the state of dentition and the location of fracture can be made. The association seemed to be more non-random in jaws with individual teeth missing and in fully dentulous jaws than it was in edentulous, quadrants of teeth missing, or deciduous and mixed dentition categories of jaws. Furthermore, it is suggested, with reservations, that the association between all dental states and subcondylar and angle or body fractures is more non-random than is the case with chin or other fracture loci. Finally, the association between chin and other fracture sites with jaws which are edentulous, have quadrants of teeth missing, or have deciduous and mixed dentition must be considered to be random.

RESULTS

The association between the state of dentition and the location of the fracture is presented in Table 1. On the basis of percentages alone, the relation between edentulous jaws and fracture location appeared significant, for, in this group, 61 per cent of the fractures were in the angle-body region, with only 22 per cent in the subcondylar area. In dentulous jaws, more fractures were found in the subcondylar than in the angle or body area. There appeared to be no difference in the frequency of subcondylar and angle or body fractures in the individual teeth missing or in the quadrants missing groups. However, these percentages are misleading, as indicated below by the results of the chi-square test.

The null hypothesis for the data in Table 1 has been given above (see "Materials and Methods"), and this hypothesis has been refuted. Additional chi-square tests on

TABLE 2

RELATION BETWEEN SITE OF TRAUMA AND LOCATION OF FRACTURES (PER CENT)

Site of Trauma	Fracture Location											
	Subcondylar			Angle or Body			Chin			Other		
	Edentulous	Decid. and Mixed	Quads. Missing	Edentulous	Decid. and Mixed	Quads. Missing	Edentulous	Decid. and Mixed	Quads. Missing	Edentulous	Decid. and Mixed	Quads. Missing
Chin point	58	75	54	25	6	14	17	19	11	.....	.....	21
Lateral chin	20	22	36	60	45	36	.....	33	14	20	.....	14
Chin area	.....	33	50	100	.....	38	.....	.....	.....	.....	67	12
Angle or body	13	26	33	70	64	61	9	.....	6	9	10	.....
Unknown	.....	25	50	80	25	50	.....	50	.....	20	.....	.....

select associations between the dental state and fracture location have, as indicated previously, demonstrated that certain associations by themselves are non-random.

First of all, it seemed to be quite apparent that mandibles which were fully dentulous or lacked individual teeth had the most frequent non-random association with fractures. To a lesser degree, there was a strong possibility that, regardless of the state of the dentition, the most non-random and common fractures were in the subcondylar or the angle-body region of the jaw. Fractures of jaws which lacked dentition or contained deciduous and mixed dentition or had quadrants of teeth missing had no predictable pattern for fractures. The location of fractures at the chin or in other regions as related to all states of dentition likewise was a random event.

It is interesting to note that, in Table 1, the highest observed frequencies by columns and by rows coincided in one cell in two cases. The one cell was that which associated subcondylar fractures with jaws lacking individual teeth; the second cell was that in which angle or body fractures were associated with dentulous jaws.

Table 2 presents data concerning the relation between the site of trauma and fracture location. Each of the sample sizes for edentulous, deciduous and mixed dentition, and quadrants missing were too small to allow for application of the chi-square test.

Therefore, this test was applied only to the dentulous and individual teeth missing groups (Tables 3 and 4).

Percentages in Table 2 indicate that impacts to the chin point, irrespective of the state of dentition, produced subcondylar fractures in over half the cases hit on the chin point. Impacts to the lateral chin region, slightly off the mid-line, produced angle or body fractures in approximately one-third to two-thirds of the individuals, more angle-body fractures being produced due to lateral chin blows in edentulous individuals

TABLE 3*

ASSOCIATION BETWEEN SITE OF TRAUMA AND FRACTURE LOCATION IN DENTULOUS JAWS

SITE OF TRAUMA	FRACTURE LOCATION				TOTAL
	Angle or Body	Subcondylar	Other	Chin	
Angle or body	32.79 +0 47 (62)	23.53 10 (13)	10.03 + 15 (20)	9.64 4 (5)	76
Chin point	12.51 4 (14)	8.98 0 18 (62)	3.83 3 (11)	3.68 4 (14)	29
Chin area	9.92 2 (9)	7.12 0 11 (48)	3.04 4 (17)	2.92 6 (26)	23
Lateral chin	23.73 0 24 (44)	16.93 + 19 (35)	7.26 3 (5)	6.98 + 9 (16)	55
Unknown	6.04 0 8 (56)	4.34 3 (21)	1.85 1 (7)	1.78 2 (14)	14
Total.....	85	61	26	28	197

* $N = 197$ [113 cases]; D.F. = 12; $X^2 = 48.768$; $P = 32.909$ (0.001), 26.217 (0.01), 24.054 (0.02), 21.026 (0.05).

than in any other group. In the deciduous and mixed category, one-third of all fractures due to lateral chin impacts were found in the area of the impact, that is, in the chin region. With impacts to the angle or body region of the mandible, there was a noticeable decrease in subcondylar fractures associated with a high percentage of angle-body fractures in all the various states of dentition.

In regard to correlations between fracture site and location of trauma in the dentulous (Table 3) and the individual teeth missing groups (Table 4), the observed chi-square value was greater than the expected value at the 0.001 level of probability.

In that the null hypothesis has been more than satisfactorily refuted, the statement can be made that there was a significant association between the site of trauma and

the location of the fracture in dentulous jaws. Thus this association would arise by chance alone in less than one fracture case out of a thousand randomly selected from the population (Table 3).

Turning now to an intensive analysis of the associations within Table 3, plotting of the highest observed frequencies for each column and row indicated a clear-cut pattern. The highest columnar observed frequencies were situated in the rows for the "Angle or Body" and "Lateral Chin" categories. The association of these two trauma

TABLE 4*

ASSOCIATION BETWEEN SITE OF TRAUMA AND FRACTURE LOCATION IN JAWS WITH INDIVIDUAL TEETH MISSING

Site of Trauma	Fracture Location				Total
	Angle or Body	Subcondylar	Chin	Other	
Angle or body	27.04 +0 41 (55)	29.67 23 (31)	13.52 6 (8)	3.76 + 4 (5)	74
Chin point	15.35 4 (10)	16.84 +0 27 (64)	7.68 9 (21)	2.13 2 (5)	42
Chin area	8.77 3 (12)	9.62 0 12 (50)	4.35 9 (38)	1.22 0 (0)	24
Lateral chin	14.62 0 15 (38)	16.04 12 (30)	7.31 + 10 (25)	2.03 3 (7)	40
Unknown	6.21 0 9 (53)	6.82 5 (29)	3.11 2 (12)	0.86 1 (6)	17
Total.....	72	79	36	10	197

* $N = 197$ [101 cases]; D.F. = 12; $X^2 = 41.987$; $P = 32.909$ (0.001), 26.217 (0.01), 24.054 (0.02), 21.026 (0.05).

sites with all fracture loci was quite significant; with three degrees of freedom, $P = 11.345$ (0.01) and 16.268 (0.001), and the chi-square value was 17.235.

The highest observed row frequencies were confined to the "Angle or Body" and "Subcondylar" columns. A chi-square test on the cells concerned yielded a value of 39.149, and, with four degrees of freedom, $P = 13.277$ (0.01) and 18.465 (0.001). Thus in dentulous mandibles, regardless of the site of trauma, there was a very significant non-random association between angle or body and subcondylar fractures.

A third chi-square test for the association between the cells contained in the residual columns and rows did not refute the null hypothesis. The chi-square value was 0.079; the degrees of freedom were 2, at which level $P = 0.040$ (0.98) and 0.103 (0.95). It is

of interest to note that the only cell in which the highest observed row and column frequency coincided was that associating angle or body trauma sites with angle or body fracture loci.

From the three chi-square tests just discussed, certain inferences are justified. First of all, it was clear that, as regards dentulous jaws, the angle or body region and the lateral chin areas in some way served more effectively to produce a fracture at some point in the jaw. The chin point, chin area, and unknown impact sites, however, served to produce fractures only in a chance manner. Furthermore, it was also apparent that in considering jaws with full dentition, the angle-body, and subcondylar regions were the most susceptible areas for fracture. The chin and other regions had no such predictable "weakness."

The results of the test for significant association between fracture location and site of trauma in patients with individual mandibular teeth missing were statistically significant at the 0.001 level of probability (Table 4).

After plotting the cells with the highest observed frequencies by row and column, the distribution suggested that chi-square analyses be made of the angle or body and subcondylar fracture loci and of the angle or body, chin point, and lateral chin trauma sites.

The chi-square analysis of the angle or body and subcondylar fracture sites in jaws with individual teeth missing, regardless of the trauma site, was impressive. The chi-square value was 28.719; at four degrees of freedom, $P = 13.277$ (0.01) and 18.465 (0.001). This more than satisfactorily refuted the null hypothesis. As was the case when a similar analysis was conducted on Table 3, it was apparent that the association of angle or body and subcondylar fractures in jaws with individual teeth missing was not a chance event, again suggesting that these two regions of the mandible were very susceptible to fractures.

The chi-square test on the above-mentioned trauma sites also refuted the null hypothesis. In this case, chi-square = 29.336, and, with six degrees of freedom, $P = 16.812$ (0.01) and 22.457 (0.001). Thus the association between the trauma sites—angle or body, chin point, and lateral chin, regardless of where the fracture was produced—was very non-random in jaws with individual teeth missing. This set of associations was exactly what was found in dentulous jaws (Table 3), with the addition of the chin point trauma site. Again one can infer that these two and possibly three regions played an important role in producing mandibular fractures.

Applying the chi-square test to the residual cells of Table 4, the value was 3.272; with one degree of freedom, $P = 2.706$ (0.10) and 3.841 (0.05); thus the null hypothesis was not refuted, and the associations were considered to be random. The cells in which the highest observed column and row frequencies coincided were those which associated the chin point trauma site with subcondylar fractures and angle or body trauma sites with angle or body fractures.

There is justification in asserting that, for jaws with individual teeth missing, the angle or body and the subcondylar regions are predictable as being the expected areas for jaw fractures. The chin or other regions had no explanation for being fracture sites other than by chance. The angle-body, chin point, and lateral chin impact sites can be expected to produce a fracture somewhere in the jaw; but this was not the case when the impact site was the chin area or some unknown region.

The chi-square analyses of Tables 3 and 4 suggested various parallels and differences. Regardless of whether or not a jaw is dentulous or has individual teeth missing, fractures of the angle or body and the subcondylar regions can be expected to be produced by chance less than once in a thousand cases, regardless of the point of trauma. Furthermore, in jaws which are fully dentulous or lack individual teeth the probability of a fractured jaw when a blow is delivered to the angle or body or the lateral chin regions (regardless of where the fracture may actually occur) is quite high. That is to say, chance as the cause of this event is less than one in one thousand cases. In jaws with individual teeth missing, chin point traumas may be expected to produce equally non-random fractures.

In addition to these observations, it should be noted that the highest observed frequencies which coincided in terms of sites of traumas and fracture loci in both tables

TABLE 5*

ASSOCIATION BETWEEN STATE OF DENTITION AND NUMBER OF FRACTURES

No. of Fractures	Dentition					Total
	Deciduous and Mixed	Dentulous	Quadrants Missing	Edentulous	Individual Teeth Missing	
1	14.00 + 20 (14)	54.55 +0 58 (38)	22.21 + 23 (35)	14.48 9 (3)	48.76 44 (9)	154
2	11.82 4 (6)	46.05 0 50 (38)	18.75 12 (28)	12.23 + 18 (13)	41.16 + 46 (15)	130
3–6	3.18 5 (9)	12.40 5 (14)	5.05 0 11 (31)	3.29 3 (14)	11.08 0 11 (31)	35
Total.	29	113	46	30	101	319

* $N = 319$; D.F. = 8; $X^2 = 29.085$; $P = 26.125$ (0.001), 20.090 (0.01), 18.168 (0.02), 15.507 (0.05).

appeared in the cell associating angle or body trauma sites with angle or body fractures. Additionally, in jaws lacking individual teeth there was a similar association for the cell relating chin point traumas with subcondylar fractures. These similarities might suggest that the angle or body region of the mandible was quite liable to fracture when that area was the point of impact.

The chi-square analysis of the state of dentition and the number of fractures (Table 5) indicated a non-random association, in that the chi-square value exceeded the expected value at the 0.001 level of probability. Once more a plot of the highest observed frequencies by rows and columns indicated a regularity. Here the highest observed frequencies for the columns appear in the rows for single and double fractures. Applying the chi-square test yielded a value of 15.797; with four degrees of freedom, $P = 13.277$ (0.01) and 18.465 (0.001). The null hypothesis was not refuted,

but there is a suggestion that the association between single and double fractures was not random.

There is a striking regularity in the identical location of the highest observed values by rows and columns within the table. This, with the suspect refutation of the null hypothesis just presented, we feel adds support to our inference that the number of fractures is significantly associated with the various states of dentition but that particular states of dentition cannot be singled out as being more non-random in this association than in others. Obviously, fractures on the order of three to six in a single

TABLE 6*

ASSOCIATION BETWEEN SITE OF TRAUMA AND NUMBER OF FRACTURES

SITE OF TRAUMA	NUMBER OF FRACTURES				TOTAL
	1	2	3	4–6	
Angle or body	58.92 +0 67 (52)	40.61 + 45 (36)	23.49 12 (9)	3.98 3 (2)	127
Chin point	29.23 0 29 (47)	20.14 10 (16)	11.65 + 20 (32)	19.75 + 4 (6)	63
Lateral chin	31.85 23 (34)	21.42 0 27 (40)	12.39 16 (24)	2.10 1 (2)	67
Unknown	12.54 0 14 (52)	8.63 8 (30)	4.99 4 (15)	0.85 1 (4)	27
Chin area	16.24 0 15 (43)	11.19 12 (34)	6.47 7 (20)	1.10 1 (3)	35
Total.....	148	102	59	10	319

* $N = 319$; D.F. = 12; $\chi^2 = 26.442$; $P = 32.909$ (0.001), 26.217 (0.01), 24.054 (0.02), 21.026 (0.05).

mandible simultaneously should require either a massive impact or multiple impacts. The only cell which was the intersect for the highest observed value for a particular column and row was the cell associating a single fracture with dentulous jaws.

The association between the sites of trauma and the number of fractures was high, the chi-square value being between the 0.01 and 0.001 level of probability (Table 6). Detailed inspection of Table 6 reveals that those cells with the greatest observed frequencies for each column are grouped within the top two rows, which designate the "Angle or Body" and "Chin Point" trauma sites. Those cells with the greatest observed frequency for each row of Table 6 are grouped within the two columns which designate 1 and 2 fractures.

Application of the chi-square test to the "Angle or Body" and "Chin Point" trauma sites demonstrated that the null hypothesis was satisfactorily refuted. The total of the chi-squares for this test was 20.197; with three degrees of freedom, $P = 11.345$ (0.01) and 16.268 (0.001). Thus the association between the angle or body and chin point regions as the sites of trauma, regardless of the number of fractures produced, was very orderly and non-random.

Application of yet another chi-square test to the association between 1 and 2 fractures, regardless of the trauma site, did not yield a chi-square value which was large enough to refute the null hypothesis. Here chi-square = 7.662, and, with four degrees of freedom, $P = 7.779$ (0.10) and 9.488 (0.05). Thus the null hypothesis for this test —that the number of fractures is not associated for causes other than chance, regardless of the trauma location—was not indicated as being a false assumption.

Two additional chi-square tests of the residual cells in Table 6 did not approach a magnitude which threatened or rendered suspect the null hypotheses involved. There is only one cell in Table 6 in which the highest observed value for a particular column was also the highest observed value for the same row. This is the cell associating one fracture with the angle or body site of trauma.

Two chi-square tests were made to ascertain non-randomness of the association between age by decades and the number of fractures, and the association between age by decades and the location of fractures. Neither of these tests proved significant at or above the 0.01 level of probability. Thus, in each of these two cases, the null hypothesis was sustained, and the associations were due to random causes only.

DISCUSSION

There was a significant association between the state of dentition and the location of the fracture which was not random. In this light it was noted that, regardless of the fracture site, the association between jaws with individual teeth missing and fully dentulous jaws was not due to chance. With some reservations, this was also found to be true for the relationship between subcondylar and angle or body fracture sites, regardless of the state of dentition.

The association between the site of trauma and the location of fracture was demonstrated to be due to factors other than chance in dentulous jaws. Furthermore, in dentulous jaws the relationship between angle or body and lateral chin impacts was non-random, regardless of the fracture site; this was not true as regards the chin point, chin area, and unknown regions of the jaw. In dentulous jaws when the trauma location was ignored, there was a significant association between angle or body and subcondylar fractures, but not for chin and other areas.

The null hypothesis was refuted when the association between the trauma site and the location of fracture in jaws with individual teeth missing was analyzed. In these jaws, regardless of the locus of the trauma, there was also a non-random association between the angle or body and subcondylar fracture sites, while the chin and other areas were randomly seen areas for fractures. Finally, regardless of the fracture location, the association between angle or body, chin point, and lateral chin trauma sites was not found to be attributable to chance. The reverse was true for the chin area and unknown impact sites.

The result of the chi-square test when applied to the state of dentition and the

number of fractures indicated that the null hypothesis was not acceptable. With reservations, additional analysis indicated that, regardless of the state of dentition, the association between single and double fractures was due to factors other than chance alone but that the association between dental state and three to six fractures was attributable only to chance.

The site of the trauma and the number of fractures produced proved also to be nonrandom. The association between the angle or body and chin point trauma sites was not a chance occurrence, regardless of the number of fractures. Chance alone was a sufficient explanation for the lateral chin, unknown, and chin area impact site association with the number of fractures.

Certain regularities became apparent when the results of the various chi-square tests were compared. One of these was that, in dentulous jaws and mandibles with individual teeth missing, the angle or body and subcondylar fracture sites association was significant, while chance alone explained the other fracture sites (regardless of the location of the fractures). This same association (or lack of it) was tentatively accepted, regardless of the dental state. There was a significant association between the angle or body and lateral chin sites of trauma, regardless of the fracture site, both in dentulous jaws and in jaws with individual teeth missing, but this was not true for the other trauma sites. In regard to the latter dental state, this was also true of the chin point trauma site, which paralleled the association between the angle or body and chin point trauma site association, regardless of the number of fractures.

One parallel is evident when the individual cells which combined the highest observed frequencies by rows and by columns are compared. This is found in the association between angle or body sites of trauma and angle or body fracture loci in dentulous mandibles and jaws with individual teeth missing.

From the above, it is apparent that the angle or body and the subcondylar regions of the mandible were most susceptible to fractures, for this association was demonstrable regardless of the dental state and regardless of the site of the trauma in dentulous jaws and in jaws with individual teeth missing. Again the angle or body region of the jaw had an association with the lateral chin and the chin point as a significant site of trauma, regardless of where the fracture was produced or the number of fractures; this may be limited to dentulous mandibles or to those in which individual teeth are missing.

Finally, there is some suggestion that angle or body trauma sites had a high association with angle or body fractures on the basis of individual cells with the highest observed frequencies for rows and columns. The authors hasten to point out that this last statement should be investigated in much more detail, since there is no statistical proof of its validity.

Associations between age of the individual, by decade, and the location of the fracture and that between age and the number of fractures per case are not statistically significant.

SUMMARY

Statistical analysis of 319 case histories of mandibular fractures revealed certain significant associations. For analysis, the data were grouped according to the state of dentition, by the site of impact, by the location of the fractures, and by the number of

fractures per case. There was a significant association between the dental state and the fracture location. With respect to dentulous jaws, the association and the state of trauma and the location of fractures was proved to be due to other than chance factors. Jaws with a few scattered teeth missing demonstrated that the trauma site and the location of fracture were not due to chance but could be predicted in their occurrence. The state of the dentition and the number of fractures were likewise orderly and not chance events. The trauma site and the number of fractures produced also were non-random. Associations between age of the individual by decade and the location of fractures and that between age and the number of fractures per case proved to be due to chance alone.

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International Association for Dental Research

*Scientific Proceedings of the British Division**

Compiled by E. D. FARMER, *Editor, British Division*

1. SENSATION IN HUMAN DENTIN.—*D. J. Anderson and M. N. Naylor, Guy's Hospital Medical School, London, S.E. 1.* Previous experiments (Anderson *et al., J. D. Res.*, **37**:669, 1958) showed that pain could be evoked by applying concentrated sugar solutions to human dentin. Acetylcholine, 10^{-2} gm/ml IM KCl did not cause pain, although these substances had been shown to be capable of doing so when applied in very dilute solutions to exposed cutaneous nerve endings (Armstrong *et al., J. Physiol.*, **120**:326, 1953). Further experiments were reported using a variety of solutions with osmotic pressures in the range of approximately 25–2,500 atmospheres. Those with high osmotic pressures were the most successful in producing pain. Specific pain-producing substances failed to cause pain when applied to dentin, although most of them were successful when applied to the exposed pulp. Using teeth to be extracted for orthodontic purposes, substances with high osmotic pressures were placed in freshly cut cavities, and the teeth were extracted and fixed. No histologic evidence was obtained of disturbance in the odontoblasts or the pulp which might suggest possible mechanism of pain production in these experiments.

2. DYE DIFFUSION IN HUMAN DENTIN.—*G. A. Ronning,† Guy's Hospital Medical School, London, S.E. 1, and the University of Oregon Dental School, Portland, Oregon.* The pain evoked from human dentin by solutions with a high osmotic pressure may be due to stimulation of a receptor mechanism by a sudden fluid disturbance set up in the dentinal tubules. Observations on the diffusion of dyes were made in an attempt to determine the factors influencing fluid movement in the dentinal tubules in freshly extracted sound premolar teeth. When dyes were allowed to diffuse outward from the pulp or inward from an exposed surface of dentin, it appeared that the tubules opening on to a cut surface did not permit dye movement across this surface, although tubules opened by fracturing the dentin allowed dye diffusion both outward and inward. The barrier to dye diffusion extended for only a short distance from the cut surface and was probably due to alterations of the protein content of the tubules, since it can be produced in tubules opened by fracturing if the dentin surface is exposed to protein precipitants or heat. It can occur when cavities are cut into the dentin even with slow-running cooled burs, and in vivo it may be a factor influencing the sensitivity to solutions applied to the dentin.

3. TILTING MOVEMENTS OF TEETH DURING BITING.—*D. C. A. Picton, Nuffield Research Fellow, University College Hospital Medical School, London, W.C. 1.* Movements of a tooth in its socket, relative to the adjacent teeth, can be detected with high-compliance resistance-wire strain gauges. Two such transducers were fixed to a rubber dam clamp on the test tooth. A pointer at the free end of each strain gauge was adjusted to rest on the neighboring tooth. Movements of the clamped tooth

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* The British Division held its ninth meeting at the University of Bristol on Thursday and Friday, April 6 and 7, 1961. The meeting was attended by 139 members and guests. Forty-nine papers and seven demonstrations were presented. The Colgate-Palmolive prize awarded for the best contribution by a person under thirty-five years of age was awarded to M. N. Naylor, of Guy's Hospital Dental School.

† Introduced by D. J. Anderson.

caused distortion of the gauges, resulting in a proportionate change in resistance. If the strain gauges were placed occlusally, to bear mesially and distally, then anteroposterior tilt of the test tooth was detected. The movements of sixty teeth were studied in ten subjects. Force was applied by the opposing teeth through a dynamometer, 2.5 cm. high, to the selected tooth. Movements were recorded when a 5-kg. thrust was exerted on the test tooth, a distal tooth, and a mesial tooth. All the forty premolars and first molars examined, with one exception, were found to tilt mesially when under load. Of the twenty upper central incisors, sixteen tilted mesially and four distally when a force of 2 kg. was applied to the center of the incisive edge. When force was directed on any distal tooth, all cheek teeth anterior to this were tilted mesially, provided that adjacent teeth were in contact. A slight mesial displacement of the upper central incisors was found in all but two quadrants when force was applied to a cheek tooth. Biting force on a canine or first premolar caused a slight mesial tilt of the second premolar but little or no displacement of the first molar.

4. The Relationship between Unimpeded Erupted Rates and Blood Pressure in the Rat Incisor.—*J. H. P. Main, School of Dental Surgery, University of Edinburgh, Scotland.* The unimpeded eruption rate of the lower left incisor was measured in 40 Wistar rats of both sexes aged about 4 months. The arterial blood pressure was measured at the same time by means of an indirect method, using the tail. When the mean unimpeded eruption rates had been established, one-third of the rats were given hydralazine, one-third were given guanethidine (both intramuscularly), and the remaining third served as controls. This was continued daily for 12 days, with increasing doses of up to 16 mg/kg of hydralazine and 15 mg/kg of guanethidine. The drugs were then withdrawn and measurements continued for a further 8 days. No significant differences were found between the eruption rates of these groups, with the exception of the hydralazine group, in which, during the period of administration of the drug, the eruption rate was 0.1 mm/48 hrs lower than at other times. This gave a *t*-value of dubious significance, and the change was in the opposite direction to that expected on the basis of the blood pressure hypothesis. The blood pressure was ascertained by 3 hourly measurements over a period of 24 hours in 10 rats. Guanethidine was found to lower it by about 15 per cent and hydralazine by 23 per cent. Using a method involving a pressure chamber with transparent walls and direct microscopy, capillary pressure was measured, and it was shown that guanethidine raised the mean capillary, or small vessel, blood pressure.

5. Solubility of Enamel *in situ*.—*C. P. Wallis, University of Edinburgh, Scotland.* The rate of solution of phosphate from the surface of teeth in the mouth was determined by a modification of the method of Marthaler and Zilio (*Helv. odont. Acta*, **4**:27, 1960) using a lower acidity and eliminating subjective measurement. The tooth was polished with a fine abrasive, isolated, and dried. 1.4 μl. M citrate buffer pH 3.5 containing 2 per cent Orange G was taken up on a 3-mm. disk of Whatman No. 4 paper. The disk was placed on the tooth surface for 60 seconds, then extracted with 3 ml. water, and the optical density of the solution was measured at 480 mμ. One ml. of reagent containing 0.8 per cent ammonium molybdate, 4 N sulfuric acid, and 0.02 per cent hydrazine sulfate was added. The solution was heated in a boiling water bath for 15 minutes, cooled thoroughly, shaken with 1 ml. cooled 4-methyl-2-pentanone (Rhodes, *Nature*, **176**:215, 1955), and centrifuged briefly. The optical density of the upper layer was measured at 780 mμ in a 20 $\times$ 3-mm. cell. Dye and phosphate standards and phosphate blanks were run at the same time, and the recovery was calculated from the dye concentration. The range of the analytical method is 0.5–10 millimicromoles of phosphorus, corresponding approximately to an enamel thickness of 10–200 Angstrom units.

6. The Variation of the Temperature of the Gingiva under Conditions of Primary Occlusal Traumatism.—*R. M. Browne, Department of Dental Surgery, University of Birmingham.* Temperature determinations were made upon rabbits with a thermocouple system (Browne, *J. Physiol.*, **152**:48P, 1960), the feeler thermojunc-

tion of which was placed in the gingival crevice. Nine rabbits served as controls. The difference between simultaneous temperature measurements upon the labial gingiva of the two upper first incisors varied from day to day. The mean range of temperature difference in the control animals was 0.69° C. In 13 rabbits a high crown was cemented to one central incisor. No significant fluctuation of the temperature difference beyond that of the controls was found in the experimental animals unless the crowns had been borne for more than 3–5 days, after which the gingiva of the experimental tooth exhibited either a relative temperature increase or decrease. The mean range in temperature difference in the experimental animals was 0.89° C. Histologic examination of the teeth and their surrounding structures revealed the effects of severe trauma in the periodontal membranes of those crowned teeth which exhibited a relative temperature decrease. The effects of milder trauma were present in the periodontal membranes of those crowned teeth that exhibited a relative temperature increase. The evidence suggests that in the former group the periodontal vessels were completely occluded, thus reducing the gingival blood supply. In the latter group the increased masticatory movement of the tooth in its socket may have acted rather as a circulatory stimulant.

7. Cold Sensation in Human Dentin.—*M. N. Naylor, Department of Dental Medicine, Guy's Hospital, London, S.W. 1.* Anderson *et al.* (*J. D. Res.*, **37**:669, 1958) showed that, while dentin is sensitive to a wide variety of physical and chemical stimuli, ice-cooled water is one of the most effective. An investigation was undertaken to determine whether the receptor mechanism for pain sensation due to cold lies in the dentin or whether the cold stimulus is conducted physically, as in skin, through the dentin to excite receptors situated in the pulp. Experiments were carried out on sound, freshly extracted human teeth to study the thermal pattern at the pulpodentinal junction resulting from physical transduction of a 10° C. stimulus applied to the floor of a cavity extending into dentin. Temperature records at the pulpodentinal junction were made with a thermistor and a Wheatstone Bridge circuit. Using cavities of varying depths, a series of such experiments was conducted which enabled graphs to be plotted relating the resulting thermal pattern with the thickness of dentin separating the cavity and the pulp. In vivo experiments were carried out in which the *reaction time* was determined to a 10° C. stimulus applied to the dentin floors of cavities of varying depths prepared in sound malposed teeth. After extraction, longitudinal sections were made passing through the pulp horns, thus enabling the distance between the cavity and the nearest part of the pulp to be measured. The results of these experiments suggest that the mechanism for cold sensation does not depend on a simple physical flux but requires a special receptor in dentin.

8. Some Observations on the Morphology and Histology of the Horny Teeth of the Hagfish (*Myxine glutinosa*).—*Jennifer A. Dawson, London Hospital Medical College.* *Myxine glutinosa* is a living representative of a once numerous group of jawless fish—Aquatha—which are ancestral to modern fish. With the absence of true jaws, there is a trough-shaped dental cartilage in the floor of the mouth which supports on either side a pair of non-calcified horny tooth plates. These may be extruded from the mouth as a single unit. The tooth plates can be retracted powerfully into the mouth, producing a strong rasping action. Beneath the horny outer covering of the tooth plates is a well-defined layer of cells—the subkeratinous lamina. The nature of this layer is described by Warren (*Quart. J. Micros. Sci.*, **45**:631, 1902), who proposed that it is ontogenetically a successional tooth row. This opinion is generally accepted. Histologic and histochemical studies demonstrate a definite structure to this layer compatible with its having a supporting function for the horny ectodermal layer and also show it to be ectodermal. It is possible that this subkeratinous lamina may originally have been a successional tooth but has gradually evolved to form an additional supporting structure prolonging the life of the horny outer covering and enabling it to withstand the stresses to which the teeth are subjected. This does not preclude the possibility that the layer eventually forms a successional tooth.

9. A Study of the Oral Flora of the Cynomolgus Monkey.—*W. H. Bowen, Department of Dental Science, Royal College of Surgeons, London, W.C. 2.* Unstimulated saliva was removed, by the use of a sterile Pasteur pipette, from each of six monkeys under general anesthesia. Dark-ground microscopy, Gram's stain, and standard culture techniques were used to identify the following groups of mico-organisms in the saliva: spirochetes, streptococci, *Veillonella,* diptheroids, *Neisseria, Fusobacterium,* lactobacilli, *Candida,* coliforms, H_2S producers, salt-tolerant micrococci, and *Bacteroides.* For quantitative studies, 0.1-ml. aliquots of saliva, obtained on two separate occasions from each of the six monkeys, were used to prepare serial dilutions in heart infusion broth. The appropriate media were then inoculated as either pour or spread plates. Following incubation for 5 days at 37° C. under either aerobic or anaerobic conditions, duplicate plate counts were made of 12 bacterial categories which earlier culturing had shown to be present. High population means ($P \times 10^6$ per ml. of saliva) were given by total anaerobes 121, total aerobes 88, total streptococci 31, aerobic diphtheroids 10, *Neisseria* 5, *Fusobacterium* 4, *Veillonella* 2.7, and coliforms 1.9. Lower counts ($P \times 10^4$ per ml. of saliva) were given by salt-tolerant micrococci 14, lactobacilli 12, H_2S producers 2, and *Candida* ($P \times 10^3$) 3.

10. Some Morphological and Histochemical Features Associated with Secondary Tumors in Bone.—*N. R. Thomas, Department of Dental Science, Royal College of Surgeons, London, W.C. 2.* Histologic examination of a large series of human secondary tumors in bone has revealed the frequent presence, in tissue adjoining the metastatic deposit, of areas containing few cells and having an amorphous appearance. Accompanying findings were focal bone necrosis, other changes in the osseous tissue, and zones of intertrabecular fibrillar connective tissue poor in cells. Cartilage plates at osteochondral junctions also appeared damaged. Metachromatically staining cancellous bone often showed the loss of its fibrillar structure when examined with polarized light. Histochemical staining of the amorphous areas with alcian blue, toluidine blue, mucihaematein P.A.S., and benzidine showed that acid mucopolysaccharides were absent from many of the areas but were present in small amounts in others. Positive reactions were given by other tissue elements known to contain acid mucoplysaccharides, e.g., cartilage and newly formed bone, showing that the demineralization required for histologic purposes had not completely removed the polysaccharide constituents. Clinical records showed a close correlation between the occurrence of areas containing acid mucopolysaccharide and previous radiotherapy, and it was considered that these hyaline areas were a manifestation of radiation damage to bone. The structureless areas free from acid mucopolysaccharide, on the other hand, gave a strongly positive benzedine reaction and were considered to be coagulated blood.

11. The Dental Follicle.—*R. W. Fearnhead, London Hospital Medical College.* The histologic differentiation of the dental follicle surrounding tooth germs of 14 human fetuses with an age range of from 6 weeks to 7 months, was studied microscopically in serial sections stained with hematoxylin and eosin, Masson's trichrome, and a modified Holmes's silver impregnation method after fixation in 10 per cent N.F.S. and Bouin's solution. In the early stages of tooth-germ development, the undifferentiated mesenchyme surrounding the tooth bud could not readily be defined as follicular tissue except for a narrow zone of cells closely adapted to the primordium of the outer enamel epithelium and the base of the dentin papilla. As tooth-germ development proceeded, well-marked regional differentiation of the follicle appeared between the tooth germ and the walls of the bony crypt. Nerve fibers entering the follicle appeared to be in two groups, one confirmed to the outermost part of the follicle near the crypt walls and the other closely applied to the outer enamel epithelium of the developing enamel organ.

12. The Blood Supply to the Rodent Incisor Periodontium.—*D. Adams, Anatomy Department, Edinburgh University, Edinburgh, Scotland.* The vascularity of the tissues immediately surrounding the incisors was examined by means of India

ink and radio-opaque perfusions in hamsters, rats, and mice. Over the enamel organ long slender arterioles ran in the long axis of the tooth to supply large capillaries, which lay in troughs between the folds of the external enamel epithelium. The transverse arrangement of these folds, described by Reith (*Anat. Rec.*, **133**:75, 1959) in the rat incisor, was similar in all the rodents examined. Venous drainage of the capillary network was due to a dense plexus of wide vessels which lay against the bony wall of the socket. Communication between the capillaries and veins appeared to occur only along the line of the cementum-enamel junction. The density of the capillary plexus was greatest in the region of the stellate reticulum and where the developing enamel first became acid-soluble. Since the tooth was in continuous movement, the arterial branches to the capillaries either grew in length or migrated along the plexus or remained stationary, while the ameloblasts moved forward relative to the capillary plexus. In the tissue surrounding the cementum-covered part of the tooth, the vessels formed a looser plexus situated nearer the bone than the tooth. Since they lay peripheral to the "intermediate fibrous plexus," it was thought that these vessels did not undergo growth changes in association with the eruptive movements of the tooth.

13. A Histochemical Study of the Intertubular and Peritubular Matrices in Normal Dentin.—*N. B. B. Symons, Dental School, University of St. Andrews, Dundee.* Serial ground sections of sound teeth were stained with 0.0005 M methylene blue, 0.01 per cent toluidine blue, and 0.05 per cent alcian blue at pH 2.6–7.0, and the P.A.S. method. With methylene blue at pH 2.6 and 3.6 the calcified dentin showed three zones. The innermost zone, 20–25 μ wide, immediately adjacent to the predentin, was stained; it also showed intense staining with alcian blue and the P.A.S. method, and the metachromasia with toluidine blue at pH 3.6. The second zone extended 40–80 μ farther into the dentin and showed no staining with methylene blue or with toluidine blue at pH 2.6 and 3.6, and a gradual reduction in the depth of P.A.S. staining. In both zones, only intertubular matrix was present. It would appear that the first zone is rich in polysaccharide and especially acid mucopolysaccharide; it is concluded that calcification of the intertubular matrix is virtually completed in it. The third zone was produced by the appearance and staining of peritubular matrix; in it the intertubular matrix was unstained at pH 2.6 and 3.6. The peritubular matrix stained intensely and metachromatically with methylene blue and toluidine blue at pH 2.6 and 3.6 and deeply with alcian blue at pH 2.6. Observations suggest that the peritubular matrix rapidly attains a high level of calcification.

14. Alizarin Uptake in the Teeth of Intravitally Stained Rats.—*C. H. Tonge, King's College, Newcastle upon Tyne.* It is well recognized that the administration of the alizarin purpurin group of dyes selectively stains calcium deposits in tissues. Their main use has been in studies based on the examination of osteologic specimens or ground microscopic preparations. In this investigation calcium deposition in the teeth of postnatal rats as demonstrated by alizarin uptake was studied, using undecalcified microscopic sections in which the relationship of the cellular elements to the calcified tissues was shown by suitably counterstaining some sections. Rats varying in age from birth to 11 days were given a single intraperitoneal injection of 0.2 ml. of a 2 per cent sodium alizarin sulfonate (C.I. 1034) solution and killed 24 hours later. After fixation in 10 per cent neutral formalin, paraffin sections were prepared and cut without decalcification. Serial sections of about 8 μ were mounted, alternate sections being counterstained by various methods to show the tissues adjacent to the alizarin-stained areas. The enamel stained a reddish color throughout, but the greatest alizarin concentration occurred in a band about the middle of its thickness or rather nearer the surface. The dentin was mainly clear, showing only a heavily stained line at the junction of the predentin and dentin. In bone it is possible to differentiate an active area of calcification by comparing its denser-staining reaction with the relatively unstained part. If the intravitally injected specimens are decalcified, the stain is extracted. Injection of pregnant rats, although heavily staining the maternal skeleton and tissues, failed to stain the fetuses.

15. The Development of Certain Facial Sutures during Fetal Life.—*J. Scott, Anatomy Department, Queen's University, Belfast.* The following facial sutures—the mid-palatal, the transverse palatal, the zygomatico-maxillary, and the naso-maxillary—were studied in serial sections in fetal material ranging from 10 weeks to full term. The development of the various layers in the sutures, the formation of the fibrous bundles, and the vascularity of the sutures are described.

16. The Surface Polish of Thin Enamel Sections.—*J. Mathieson, Department of Physics, University of Birmingham.* Irregularities in the surfaces of a thin enamel section are important when phase-contrast or interference methods of examination are used. Thin replicas were made of enamel sections polished by various methods. Electron-microscope photographs were shown from which the best technique for polishing can be determined.

17. X-Ray Studies of Thin Tooth Sections.—*J. H. Fremlin, Department of Physics, University of Birmingham.* Thin sections made by the method described at the eighth meeting of the I.A.D.R. were examined by X-ray methods. Resolution was not so good as can be obtained by the optical microscope but gave some useful complementary information.

18. An Investigation of the Comparative Value of Different Acids for the Decalcification of Hard Tissues for Histology.—*E. B. Brain, Department of Dental Science, Royal College of Surgeons, London, W.C. 2.* An investigation was made regarding the suitability of twelve acids, each of normal concentration, for demineralization and subsequent staining for histologic purposes. The twelve acids used were nitric, hydrochloric, trichloracetic, phosphoric, formic, lactic, citric, acetic, succinic, oxalic, sulfuric, and tartaric. The histologic specimens were of approximately the same dimensions and consisted of the first and second permanent maxillary molar teeth and supporting bone, removed from monkeys which had been killed by perfusion with formalin. A specimen was placed in a specific volume of each acid at room temperature. The acid was unchanged throughout the experiment, and the process of decalcification was observed by X-ray examination. The tissues were processed by a routine method after decalcification was complete. Among the principal findings it was noted that the time required to decalcify nine specimens varied from two to seventy-five days. The oxalic, sulfuric, and tartaric acid solutions failed to decalcify the specimens completely. Differences in the staining reactions were also observed. Examination of the sections prepared from the material treated with acetic or succinic acid also showed that large amounts of enamel were preserved, especially in the interdental region. Further experiments are being undertaken to develop methods for the preparation of decalcified sections of the enamel *in situ*.

19. Experimental Studies of Enamel Epithelium.—*B. Cohen, Department of Dental Science, Royal College of Surgeons, London, W.C. 2.* Experiments have been carried out with a view to studying the properties and functions of enamel epithelium during and after tooth eruption. The enamel epithelium covering unerupted permanent teeth in young monkeys was extirpated surgically and subsequent eruption of the teeth observed. It would appear that this procedure does not interfere unduly with normal eruption, but marked changes in the gingival architecture ensue. From these and from subsequent histologic studies, it would seem that enamel epithelium in some way provides attachment of the oral mucosa to the crown of the tooth, thus determining the outline of the gingival margin. The degree to which enamel epithelium is in biological continuity with enamel was tested by passing mylar strips down the gingival crevice and making subsequent microscopic examination.

20. Downgrowth of Gingival Epithelium over the Roots of Teeth in Monkeys.—*W. D. McHugh, Dental School, University of St. Andrews, Dundee.* The histology of gingival epithelium proliferating over the root cementum was studied in 30 monkeys between the ages of 1½ and 5 years. Epithelium was present on the cementum of most deciduous or permanent teeth which had been erupted for more than 2–3 years. This epithelium was usually 6–12 cells thick, with a well-defined

basal layer next to the connective tissue and prickle-type cells between this and the cementum; flattened cells and nuclear disintegration were rarely seen. Analysis of the incidence of dividing cells showed that an appreciable proportion (13 per cent) of the total number of mitotic figures in this epithelium were in cells actually in contact with cementum. A narrow band of apparently homogeneous material was often observed between epithelium and cementum. This band did not show evidence of keratinization but was similar in thickness and staining characteristics to pre-cementum. It is concluded that epithelium in contact with cementum does not become keratinized, as suggested by Gottleib (*Deut. Z. Zahnheilk.*, **34**:142, 1921) and many others. Small areas in which the cementum was devoid of covering cementoblasts were often just apical to the tip of the epithelial downgrowth; distintegration of connective tissue fibers was observed in these areas. Some infiltration of gingival connective tissue by inflammatory cells, over 90 per cent of which were plasma cells, was found in every case. It was therefore impossible to determine whether the epithelial downgrowth was physiologic or pathologic.

21. The Ontogeny of Connate Incisors in the Dog.—*A. D. Hitchin and I. Morris, Dundee Dental School, St. Andrews University.* The inheritance in Lakeland terriers of connate incisors with a number of variants has been demonstrated (Hitchin and Morris, *J. D. Res.*, **37**:755, 1958, and **39**:1101, 1960). The ontogeny of this phenomenon was studied in serial sections from the thirty-fourth day of uterine life until 60 hours after birth. When the first and second incisor germs were united, a large secondary germ budded from the lingual side of the united germs to form a macrodont permanent tooth. If the first and second primary germs were connate on one side only, they were tied together on the otherwise normal side by a persisting band of interdental lamina. If, with separate deciduous incisors, connate secondary germs occurred unilaterally, on the other side the permanent first and second germs were united by a persisting band of lamina even at birth. The primary anatomical abnormality in connation of incisors was persistence of the interdental lamina, which is controlled by the same genes in maxilla and mandible; for, when connate primary upper incisors occurred, either the lowers were connate, or their germs were united by persisting tooth bands. The converse also held. Persisting lamina was present when the appearance of connation of an incisor of the normal series with a supernumerary occurred, which may be due to herniation of the contents of the incisor germ between the laminar layers.

22. Dental Pulp Changes Promoted by 90 Sr.—*M. A. Rushton, Guy's Hospital, London.* 600 microcuries/kg 90 Sr given by single injection to weanling rabbits caused early damage among differentiating odontoblasts and subsequent depletion of the expected number of adjacent pulp cells. Cheek teeth ceased to grow in length and became solid with defective dentin. Nodules of inactive pulp remained at the base of each lobe of tooth up to 6 months' observation. Changes in lower incisors were similar except that teeth did not cease growth in length but became narrower. Severe damage to pulp cells next to the internal enamel epithelium did not prevent provision of a continuous layer of odontoblasts. These were derived from division of recently differentiated odontoblasts and presumably of adjacent pulp cells. When older rabbits were injected at 21 months, the effects on teeth were still severe; but when done at 38 months, less so, with continued growth of teeth in length. In these, after 3 months, pulps appeared much recovered, and after 6 months nearly all defective and 90 Sr-bearing dentin was lost by attrition. Dosimetric studies by Dr. Maureen Owen on contralateral mandibles provided dose rates and accumulated doses for different time intervals and areas (Rushton, Owen, Holgate, and Vaughan, *Arch. Oral Biol.*, in press). With the possible exception of cementum, dental tissues did not give rise to the osteosarcomas regularly arising beside teeth.

23. Diagnosing Features of Pemphigus Affecting the Oral Mucosa.—*B. E. D. Cooke, Guy's Hospital, London.* Since *pemphigus vulgaris* and vegetans may affect the oral mucosa for 2 years or more before there are any skin lesions, the diagnosis has

to be made on the oral manifestations alone. Large flaccid bullae, resembling epithelium floating free, characterized the clinical picture of *pemphigus vulgaris,* and snail-track vegetations and deep fissures, pemphigus vegetans. Nikolsky sign was not always present in pemphigus vulgaris and rarely so in vegetans. A biopsy was mandatory, with epithelial smears providing valuable confirmatory evidence. In pemphigus, even when the epithelium stripped off at the slightest touch of the scalpel, it could be seen that the basal layer was left behind on the corium and that the deepest prickle cells on the epithelial tag were acantholytic. When a biopsy was taken from the mucosa adjacent to bullae, the mild trauma so entailed is enough to produce a "histological Nikolsky" with separation of the suprabasal layer. Epithelial smears are simple to obtain but are not always so reliable. True Tzanck cells with round hyperchromatic nuclei and a peripheral condensation of basophilic cytoplasm were not easily found even in the florid cases, but all transitions to normal cells were observed. Perhaps the most striking feature of these smears was the profusion of multinucleated cells, the result of the great reparative power of the oral mucosa.

24. The Laboratory Testing of Some Agents Used in Oral Surgery.—*G. Colman, Turner Dental School, Manchester 15.* Whitehead's varnish, "Biosone," Gelfoam,* zinc oxide–eugenol, and bismuth iodoform paraffin paste have been tested for antibacterial action. The separate components of these agents, except for Gelfoam, have been similarly tested. The ability to diffuse through tubes of serum agar seeded with test was the criterion used. As a measure of toxicity the agents were placed on the chorioallantoic membrane of 11-day chick embryos.

25. Field Sterilization in Endodontia.—*T. H. Melville and R. H. Birch, School of Dental Surgery, University of Liverpool.* Results of investigations into the bacteriology of root canals assume that recontamination of the canals from exogenous sources is kept to a minimum. Experiments were carried out to assess the degree of contamination of the isolated tooth surfaces and the rubber-dam field under variable operating conditions. The variables tested included (1) the method of sterilization of the equipment used; (2) the sterile clothing worn by the operator; (3) the precleaning of the tooth before application of the dam; (4) the disinfecting agent used to paint the tooth and the dam; and (5) the time for which this agent was allowed to act before sampling. After the dam had been applied to the tooth and the fields suitably prepared, bacteriologic samples were taken from 16 areas, comprising the isolated tooth surfaces and the dam. The results of these samplings were assessed by chi-square tests. Results showed that a fairly low degree of contamination existed under suitable operating conditions. A complex aseptic technique gave no significantly better results than a simple technique more easily applied under normal surgery working conditions. Adequate cleaning of the tooth under treatment prior to application of the dam showed a statistically significant reduction in the number of samples giving rise to bacterial growth; this reduction was most marked in the region of the tooth and the immediately adjoining areas of the dam.

26. Relationship of Occlusion and Toothbrushing with Caries Rates.—*J. Miller, Turner Dental School, Manchester 15.* In 700 twelve-year-old children gingival conditions were related to occlusion. It was found that in children with normal occlusion, 27 per cent had healthy gingivae, while in children with a malocclusion only 14 per cent had healthy gingivae. In the same group of children regular toothbrushing —at least once per day—was associated with better gingival conditions in children with either a normal or abnormal occlusion. Average DMF rates were found to be higher with regular toothbrushing than with irregular or no toothbrushing—the difference gave a figure for P less than 0.05. Relating occlusion to caries, children with a malocclusion had higher DMF rates than children with normal occlusion. The difference was not significant at age twelve years; but comparison of caries rates and occlusion in 268 children aged fourteen years showed that 157 children with normal occlu-

* Upjohn Company, Kalamazoo, Michigan.

sion had an average DMF of 8.27 and that 113 with a malocclusion had an average DMF of 9.49; this difference was more than three times the standard error.

27. Effect on Dental Caries of Sorbitol in the Diet of Sialoadenectomized Rats.—*R. D. Emslie and L. Sinclair, Guy's Hospital, London.* The submandibular salivary glands and infra-orbital glands of 16 albino rats were removed surgically, and the parotid ducts tied off. These animals and 7 unoperated controls were given diets containing 67 per cent carbohydrate. For 8 of the sialoadenectomized rats the carbohydrate was 50 per cent sucrose and 50 per cent starch, the other 8 having sorbitol substituted for the sucrose. The percentage of sorbitol needed to be increased gradually to this level and reduced temporarily when diarrhea occurred. The entire carbohydrate for the 7 unoperated controls was sucrose. After 4–5 months, 4 of the 8 sialoadenectomized rats on sucrose and starch had developed early caries, mainly in fissures, which was confirmed histologically. No caries was found in the other animals. This would tend to confirm the conclusion of Klapper and Volker (*J. D. Res.*, **34**:703, 1955) from studies on desalivated hamsters that sorbitol has little or no cariogenic potential.

28. Estimation of Mercury 203 in the Dental Tissues.—*G. S. Nixon, School of Dental Surgery, University of Glasgow.* Controversy has arisen from time to time over the possibility of systemic intoxication by mercury from dental amalgam through the dentin. Spectrographic analysis has shown the presence of mercury in discolored areas of dentin under dental amalgam (Massler, *J.A.D.A.*, **47**:415, 1953). In vitro experiments were carried out to determine if mercury diffusing from amalgam could be measured acurately in submicrogram quantities. The method selected was that of isotopic dilution, which consists of diluting pure mercury with one of its radioactive isotopes, in this case Hg^{203}. A known weight of this mixture was used as a standard counting source. An amalgam mix was prepared in each case, using a standard alloy with the radioactive mercury and packed into prepared cavities of similar dimensions. After varying intervals the amalgam was dislodged from the cavities. The amounts of mercury remaining in the teeth at varying distances from the cavity margins were estimated by extracting the mercury and determining its radioactivity. The results of these analyses showed that concentration of mercury Hg^{203} was greatest in dentin in immediate contract with the amalgam and, after a period of 40 days, was not found beyond a distance of 1 mm. from the original cavity margins.

29. Mechanical Properties of Stainless-Steel Wires.—*J. N. Anderson and V. P. Delgado, University of St. Andrews, Scotland, and University of São Paulo, Brazil.* Tensile properties of various grades of stainless-steel wire were determined on a Hounsfield tensometer. Values for ultimate tensile strength, proportional limit, modulus of elasticity, and elongation were obtained on specimens as received and after heat treatment, at temperatures between 200° and 600° C. for 5 minutes. Bending tests were also carried out on the original and heat-treated material. Results gave a comparison between the stainless-steel wires available in Britain.

30. The Mechanics of Cast Partial-Denture Clasps.—*J. F. Bates, Turner Dental School, Manchester.* Theoretical approach to clasp design (Warr, *Phys. in Med. Biol.*, **3**:212, 1959) has not previously been investigated other than by simple, photoelastic studies. The present work was planned as part of a more detailed study. The first part was to evaluate the physical properties, i.e., modulus of elasticity and proportional limit of gold, cobalt, and nickel-chromium alloys, using cantilever bend tests, since all previously quoted figures were for large-scale tensile test specimens. In alloys with large grains this "size effect" could produce results that were not applicable to clasps. The size and taper of some preformed wax and plasticized P.V.C. patterns is being investigated by imbedding in stone and sectioning on a Pultra slitting machine. To prevent the stone from shattering, the stone block was dehydrated and the pattern dissolved out with ethyl alcohol and then imbedded in wax. Sections (0.010″) were taken at 5-mm. intervals along the length of the pattern and the width and thickness of the clasp measured on a traveling microscope. The third part of this investigation

was the casting of these preformed patterns in straight and curved forms, with different radii of curvature, to compare the deflections at the proportional limit, since these control the depth of undercut that can be used in retention.

31. Further Studies on the Crystalline Constituents of Oral Calculus.—*S. L. Rowles, M.R.C. External Staff, School of Dental Surgery, University of Birmingham.* X-ray diffraction analysis of oral calculus has been extended to specimens obtained from persons living in several different regions of the United Kingdom. As in the case of Birmingham calculus samples previously reported, the principal constituents were hydroxyapatite, whitlockite, octocalcium phosphate, and brushite. The incidence of these calcium phosphates, however, showed some variation with the region of origin. This was particularly evident in the case of brushite, e.g., over 60 per cent in samples from Oxford compared with less than 20 per cent in Birmingham samples. In terms of relative abundance, the difference was even more marked. A few specimens of dental calculus from archeological teeth were examined. Material from Neolithic and Bronze Age teeth (from alkaline soil) contained hydroxyapatite and whitlockite. Calculus from Roman teeth also contained octocalcium phosphate.

32. The Infrared Spectrum of the Carbonate Ion in Carbonate-containing Apatites.—*J. C. Elliott, London Hospital Medical College, London, E. 1.* The exact position and shape of the absorption bands of the carbonate ion vary in different carbonate-containing substances. Elephant enamel, dahlite, and a synthetic apatite containing carbonate ions prepared as described by Ames (*Econ. Geol.*, **54**:829, 1959) were studied in the region 4,000–700 cm.$^{-1}$. The synthetic material and enamel had a very similar absorption spectrum with the carbonate band at 1,430 cm.$^{-1}$ split into a doublet but with different relative intensities. Dahlite showed a similar but smaller splitting of this band. Polarized infrared studies were made on ground longitudinal sections of human teeth. The enamel absorption spectrum showed an additional splitting of the carbonate band at 870 cm.$^{-1}$. This could be interpreted as indicating the presence of carbonate ions at right angles. The fact that both peaks at about 870 cm.$^{-1}$ showed polarization properties is difficult to reconcile with any theory involving the presence of amorphous calcium carbonate in enamel.

33. The Calcium and Phosphorus of Dental Plaque.—*C. Dawes and G. N. Jenkins, Physiology Department, Medical School, King's College, Newcastle upon Tyne, 1.* Plaque samples were collected from students, 2 days after refraining from tooth brushing and at least 3 hours after eating. The water, calcium, and phosphorus contents were determined and compared with those for salivary sediment (often used experimentally in place of plaque). The water content of sediment was about 94 per cent, while that of plaque was 80 per cent. Calculated as μg/mg of dry material, sediment contained Ca = 3.0, inorg. P = 2.5, and total P = 8.1. The amount of inorg. P can be accounted for by the above water content, but the Ca level was three times higher than expected, assuming the sediment liquid to be saliva. The average Ca value of plaque was 4.5, which is eighteen times greater than that calculated from its water content. Total P was about 16.5 μg/mg and may be fractionated into inorg. P (11 per cent), insol. organic P (64 per cent) and sol. organic P (25 per cent). The inorg. P was three to four times greater than that expected from salivary levels, and the value doubles if estimations are done on dry plaque. After a glucose rinse, inorg. P was reduced from 1.87 to 1.1 μg/mg dry plaque (estimns. on wet material). All the Ca was released by washing with buffer at pH 5.0. Analyses of plaque from eleven-year-old children gave similar values, suggesting that the high Ca and P values were not evidence of calculus formation. These values suggest that the usual figure of 5.5 for the critical pH for tooth decalcification is too high when plaque is considered.

34. The Effect of Various Anions on the Solubility of Enamel and Dentin in Acid.—*S. A. Leach and A. M. Dodge, Turner Dental School, University of Manchester.* Unless there is some specific interaction between the anion of an acid and the mineral matter of enamel and dentin, the variations in solubility that are encountered should be wholly accounted for in terms of the chemical properties of the solution and

the anion. The following acids were tested for specific interaction effects: alanine, aspartic, citric, glutamic, malic, malonic, succinic, and valine. The procedure was to estimate the decalcification in terms of calcium and phosphorus released after overnight shaking of 100 mg. of powdered enamel and dentin in 10 ml. of a buffer solution, pH 4.70, containing 0.2 M sodium acetate, 0.2 M acetic acid, 1 M sodium chloride, and each of the above acids in turn whose total acid and various anion concentrations were 0.1 M in each case. The pH of each solution at the termination of the decalcification was also measured. The buffering capacity of each solution may be calculated from the known dissociation constants of the acid and was also estimated by titrating 10 ml. of each of the unreacted solutions against 0.1 N sodium hydroxide to a pH of 5.00. With the single exception of citric acid, whose specific interaction with this mineral is already well established, the final pH of each solution and the amount of decalcification correlated with the buffering capacity of each solution as estimated above. Interaction of the various anions with the dissolved calcium in the solution was calculated to be very small and within the experimental error of the technique.

35. ORGANIC PHOSPHATES AND TOOTH SOLUBILITY.—*G. N. Jenkins, Department of Physiology, Medical School, King's College, Newcastle upon Tyne, 1.* Previous work has shown that many naturally occurring organic phosphates reduce the solubility of teeth and calcium phosphate in acetic acid buffer (pH 5.0) and in saliva incubated with carbohydrate. Of the substances tested, phytate is the most active (readily detectable in M/100,000 solution). Phytates and organic phosphates are known to be chelating agents and have been found to increase tooth solubility in some (mostly alkaline) buffers. Brown-flour extracts (which contain phytates) do not increase tooth solubility even under alkaline conditions; evidently an unidentified constituent of brown flour prevents the chelating, but not the "protective," action of phytate. Phytate is not likely to be present in the human mouth under the conditions in which it increases tooth solubility (i.e., alkaline pH and the absence of the unidentified food constituent that prevents chelation), but it might have this effect in experiments on animal caries (in which the pH of the tooth environment has usually been found to be alkaline and synthetic diets are consumed).

36. METABOLISM OF FLUORIDE IN DENTAL STRUCTURES.—*S. M. Weidmann, Biological Research Unit, School of Dentistry, University of Leeds.* Fluoride uptake was studied in growing and non-growing teeth. High amounts of fluoride were administered in order to get appreciable absorption in a relatively short space of time. Under similar conditions, up to twenty times more fluoride was taken up by the dental structures of rabbits than in cats, thus confirming the dominant role of growth in fluoride absorption. This seems to be linked up with the mechanism of the reaction. In contrast to P^{32} uptake, fluoride absorption is probably related only to the faculty of tissue to exchange its mineral ions with those of the surrounding fluids (Weidmann and Weatherall, *J. Path. Bacteriol.*, **78**:243, 1959). Newly formed hard tissues are undercalcified, and full mineralization is attained only gradually. It is quite possible that fluoride uptake is enhanced during the mineralization period, as well as during growth. Observations made on the teeth of half-grown cats seem to support this view. Considerable differences were found in the rate of fluoride incorporation between the enamel and the dentin of growing teeth. The uptake by dentin was much faster and greater than by enamel. From the over-all picture, it would appear that fluoride is incorporated into dentin by systemic reactions. For enamel, one would also have to consider surface absorption. In growing teeth, however, the major part of fluoride appears to be incorporated by systemic reactions. Retention studies showed that fluoride was equally quickly lost in growing teeth as it was incorporated, whereas no appreciable changes were seen in permanent teeth.

37. STUDIES OF ENAMEL AND BALEEN.—*F. G. E. Pautard, Department of Biomolecular Structure, University of Leeds.* The "cross-β" configuration, closely associated with the k-m-e-f (keratin-myosin-epidermin-fibrinogen) group of proteins in the supercontracted state, was observed consistently in our recent X-ray diffraction studies

of the enamel matrix of fetal ox teeth (kindly prepared by Dr. J. A. Weatherall, of the Biological Research Unit, Leeds University Dental School). These results confirmed our earlier report of the appearance of this same diffraction pattern in the enamel matrix of a decalcified unerupted human molar tooth. In contrast with the matrix from human enamel (which was imbedded at 60° C.), however, the ox fetal enamel was carefully handled at lower temperatures (2–18° C.), and it seems likely, therefore, that the "cross-β" configuration is the natural state of the protein. Other studies suggested that baleen, the keratinous epidermal whalebone of the Mystacocoeti, may be calcified. In the Fin Whale, for example, X-ray diffraction patterns of the whalebone-fringe fibers showed not only the α-diagram found in the k-m-e-f group of proteins but also reflections suggesting hydroxyapatite with the c-axis of the crystallites oriented parallel to the fiber axis. These findings were described and the analogies between enamel and baleen discussed.

38. The Composition of Collagen from the Dentin of Developing Human Deciduous Teeth.—*J. E. Eastoe, Department of Dental Science, Royal College of Surgeons, London, W.C. 2.* Incisors were removed from human fetuses (32–35 weeks *in utero*) and the calcified portions separated from surrounding soft tissues and the pulps. The enamel was fragmented and detached from the dentin by gentle scraping and subjected to analysis (Eastoe, *Nature*, **187**:411, 1960). A small amount of further material from the region of the amelodentinal junction was then removed by very vigorous scraping. The residual caps of dentin were demineralized by two successive treatments with saturated sulfur dioxide solution for 2 hours each, then washed and treated with 10 per cent sodium chloride solution for 18 hours to remove albumins. After further washing in water and acetone, the dentin collagen was dried *in vacuo* and weighed. It was hydrolyzed for 48 hours in 6 N HCl at 100°, and amino acid analysis was carried out by a semimicro method (Eastoe, *Biochem. J.*, 1961, in press). The composition was qualitatively and quantitatively typical of mammalian collagens and within the range shown by other human mesodermal tissues (compact bone, tendon, and mature permanent dentin). Close comparison of individual amino acid values showed that the developing dentin from the fetal teeth had very slightly less of those occurring in large amounts in collagen (e.g., glycine, alanine, hydroxyproline) and slightly more of those of less common occurrence (e.g., tyrosine, valine, leucines, serine) compared with collagen from mature permanent dentin. This may indicate the presence of a small proportion of mucoprotein in the developing dentin.

39. Effect of Varying Levels of Dietary Calcium and Vitamin D on the Composition of the Rat Femur.—*R. L. Hartles and A. G. Leaver, Biochemistry Department, School of Dental Surgery, University of Liverpool.* Four groups of rats were maintained for 56 days on diets of known and carefully regulated composition. Group I (control) received a diet containing 0.56 per cent Ca by weight and 0.2 mg. calciferol per kilogram. Group II received a diet similar in every respect except that the calcium level was reduced to less than 0.03 per cent. Group III received a diet identical with that of the controls except that no calciferol was included, while Group IV was fed a diet lacking calciferol and with the calcium level reduced to less than 0.03 per cent. The animals of Groups II and IV grew less than others, and the weight of femur available for analysis was only one-third that obtained from Groups I and III. It was found that the Ca and P levels were substantially reduced in both groups receiving the low Ca diets and slightly so in that deficient only in calciferol, these results being accompanied by parallel increases in nitrogen. The citric acid content, however, reacted differently. It was slightly depressed by the absence of calciferol in Group III, reduced to half its normal value in Group IV, but increased to 50 per cent above the normal in Group II, where the normal calciferol level was associated with the low level of calcium.

40. Effect of Hypervitaminosis D, in the Presence of Normal and Very Low Dietary Calcium Levels, on the Composition of the Rat Femur.—*A. G. Leaver and R. L. Hartles, Biochemistry Department, School of Dental Surgery, Uni-*

versity of Liverpool. Two experimental groups of rats were maintained on purified diets of known composition and similar to those used in the previous experiment. Experimental Group A received Ca at the normal level (0.56 per cent) and Group B at the very low level of 0.03 per cent. Both groups received calciferol at the very high level of 50 mg/kg of diet, that is 250 times the normal. The control group received 0.56 per cent Ca and 0.2 mg. calciferol per kg. of diet. Both experimental groups gained weight very poorly compared with the controls. Despite this, the weight of bone material available for analysis from the Group A animals was only slightly less than that from the controls. Analysis showed, however, a drop in Ca and P with a corresponding rise in N. The citric acid level was substantially increased to nearly twice the normal value. In group B animals the amounts of bone mineral recovered for analysis was only one-third of that obtained from the controls. Analysis showed a large drop in Ca and P and a rise in N. The citric acid level rose to a value very similar to that in Group A, though the weight of citric acid per gram of Ca was significantly greater than in Group A. A further observation was that hypervitaminosis D significantly raised the Ca/P ratio in both normal and low Ca intake.

41. Leukocytes in the Saliva of Edentulous Subjects.—*D. E. Wright, Department of Physiology, Medical School, Newcastle upon Tyne.* A series of studies is being carried out on salivary leukocytes with a view to investigating further the observation of Wright and Jenkins (*J. D. Res.*, **32**:511, 1953) that the count of intact cells in saliva specimens from caries-free subjects was approximately four times greater than in caries-actives. Recently Wright (*Brit. D. J.*, **110**:50, 1961) showed that comparatively few leukocytes are found in the saliva of infants. Sharry and Krasse (*Acta odont. Scand.*, **18**:347, 1960) reported that the main site of origin of these cells is the gingival sulcus and that in saliva specimens from 11 edentulous subjects the leukocyte count was low. The present work involved the examination of 5 samples each from 44 subjects, 20 male and 24 female, of the age range twenty-one to sixty-seven years, who had had complete upper and lower sets of dentures for at least 12 months. The intervals at which the saliva specimens were taken varied from 2 to 7 days. Subjects were requested to remove their dentures before thoroughly rinsing out their mouths with 0.5 per cent saline. Low-melting-point paraffin wax was then chewed to stimulate flow, and a specimen of saliva was collected over the period of the first 2 minutes. Out of 220 samples, 149 showed no leukocytes at all. The average count of all samples was 7.1 cells per cubic millimeter, which is much lower than the figures of 112.5 for caries-free and 31.4 for caries-active subjects quoted by Wright and Jenkins. The conclusion is therefore that, as with infants, the saliva of edentulous subjects contains relatively few leukocytes.

42. Chewing Gum as a Vehicle for Fluoride Administration Studies with F^{18}.—*R. Duckworth, R. D. Emslie, and N. Veall, Guy's Hospital, London, S.E. 1.* This study was concerned with the release of fluoride from chewing gum and its uptake by the teeth. Sodium or stannous fluoride (fluoride content 0.1 mg/gm of gum) was added to a specially formulated chewing gum. F^{18} was incorporated into the gum to give an activity of 1–2 μc/gm of gum at the time of use. The release of fluoride was followed in trained adult subjects who chewed a piece of gum weighing approximately 1 gm. for 10–15 minutes. Chewing was interrupted at 1-minute intervals to measure the activity remaining in the gum. Sodium fluoride was released rather more slowly than stannous fluoride, 90 per cent of the latter being released in less than 10 minutes. The addition of 2 per cent citric acid reduced the release of sodium fluoride. The uptake of fluoride by sound teeth of children or young adults was measured. Gum was chewed for 10 minutes immediately prior to extraction of teeth. After scrubbing each extracted tooth with soap and water for 5 minutes, the amount of F^{18} taken up by the tooth was counted. In general, one premolar tooth took up approximately 20 mμg from 10 minutes' chewing of gum containing 100 μg. of fluoride. Uptake was not materially altered by substituting stannous for sodium fluoride. The smaller release of fluoride from citrated gum was offset by the higher uptake by the teeth.

43. Acid Phosphatase Activity in the Rat Incisor Pulp Following Injury. —*H. W. Ferguson and F. E. Lawton, School of Dental Surgery, University of Liverpool.* Although the distribution of acid phosphatase in the pulps of normal human and animal teeth has been described, very little is known of its possible function. Raekallio (*Nature,* **188**:234, 1960) has recently reported the presence of the enzyme in injured skin. In an attempt to relate these findings to the dental pulp, the lower incisor of the rat was subjected to a standardized injury, and the animals sacrificed after intervals varying between 30 minutes and 48 hours. The distribution and concentration of acid phosphatase in the pulp were demonstrated by the standard Gomori lead sulfide method and by an azo-dye technique suggested by Barka (*Nature,* **187**:248, 1960) and was related to the various phases of the injury observed by normal histologic staining. Evidence was adduced to suggest that the Gomori method was not specific for enzyme activity but also stained preformed phosphate. The azo-dye technique showed that acid phosphatase was present in significant amounts within 30 minutes of injury and was related mainly to moderately injured cells. It was never demonstrated in severely damaged or necrotic tissue. Slight enzyme activity was also recorded in proliferating fibroblasts during the early phases of repair, but its function remains obscure.

44. Histochemical and Ultrastructural Evidence of Sites of Activity in Resting and Pilocarpine-stimulated Salivary Glands of the Rat.—*J. P. Waterhouse, London Hospital Medical College.* Pieces of the submandibular and parotid glands were removed under Pentobarbitone anesthesia from male Chester Beatty albino rats aged 3–7 months, before and 30 minutes after the administration of pilocarpine nitrate 15–40 mg/kg body weight parenterally. Tissues were cut freehand into small pieces and placed immediately in ice-cold fixative: 10 per cent N.F.S., T.C.A.-ethanol, Zenker's fluid, and Palade's or Dalton's solution for electron microscopy. Sections were cut at 10 μ in a cryostat or at 7 μ after paraffin imbedding. For electron microscopy they were cut with a glass knife on a heat-advance microtome after dehydration and imbedding in Araldite* or methacrylate. In the pilocarpine-stimulated submandibular gland, some crowding of the acinar cells (Shannon and Brooks, *Oral Surg., Oral Med., Oral Path.,* **13**:240, 1960) was seen. Under the electron microscope a greater part of the basal area of these acinar cells appeared occupied by endoplasmic reticulum bearing RNA granules in the stimulated than in the unstimulated gland. In the parotid gland, hematoxylin-eosin staining did not reveal a difference between stimulated and non-stimulated acinar or duct cells. P.A.S. staining, however, revealed that, whereas in the unstimulated gland reactive material occupied the whole acinar cell, in the stimulated it was limited to the apical half of the cell and was present in the duct lumens. This localized P.A.S. +ve secretory material does not contain appreciable glycogen, nor does it stain with alcian blue for acid mucopolysaccharide. The cytoplasm of the whole stimulated cell remains metachromatic with toluidine blue and reacts with D.D.D. for -SH groups.

45. The Influence of Potassium Phosphate upon Salivary Phosphorus Levels in Rats.—*S. A. Boswell and P. J. Holloway, London Hospital Medical College.* Several recent publications have suggested that an increase in the phosphate content of diets fed to experimental animals results in a reduced incidence of dental caries. The present study was an attempt to determine whether salivary phosphate levels in rats are influenced by the diet. Twenty-six-month-old male rats were fed a high-sugar, synthetic diet (diet 2700, Shaw and Griffiths, *J. D. Res.,* **39**:377, 1960). Ten of these were given an additional 10 gm. K_2HPO_4 to every 90 gm. of diet. The phosphorus contents of the two diets were 0.79 and 2.41 per cent. Pilocarpine-stimulated saliva samples were collected after 6, 34, and 44 days and estimated for phosphorus. There was a significant difference between the groups after only 6 days ($P < 0.01$): the mean for the control group was 13.8 μg P/ml saliva, and that for the

* Ciba Products Corporation, Kimberton, Pennsylvania.

experimental group was 20.3 μg P/ml. Fasting the rats for 24 hours resulted in a difference at the same level of significance, but the mean values of both groups were substantially reduced (8.2 and 16.0 μg P/ml). Although there was a significant decrease in the mean weights of the femurs from the high-phosphate rats, the percentages of calcium and phosphorus remained the same. A second experiment was carried out with three groups of rats. Additional K_2HPO_4 was added to the diet fed to two of the groups, so that the phosphorus levels in the diets were 0.79, 1.1, and 2.0 per cent. Saliva samples were collected by the same method as in the first experiment at 15, 22, 29, and 36 days. The groups receiving the diet containing 2.0 per cent phosphorus showed a significant increase in mean salivary phosphorus content when compared with the other two groups. The latter had similar salivary phosphorus levels (values after 36 days were 13.5, 13.4, and 21.2 μg P/ml). In both experiments, increases in salivary phosphorus levels were accompanied by marked loss of weight.

46. The Effect of Severe Undernutrition on the Development of the Skull and Teeth of Growing Pigs.—*W. A. B. Brown, R. A. McCance, and E. H. R. Ford, Department of Dentistry, Queen's University, Belfast; Department of Experimental Medicine and Department of Anatomy, University of Cambridge.* A study was made of the differential effects of severe and prolonged undernutrition on the growth and development of the pig's skull and teeth. For this investigation, 16 normal pigs were killed at various ages from birth to 15 months, and 18 undernourished pigs died or were killed between 3 and 15 months. After the death of the animals, lateral and occlusal radiographs were taken of the skull and teeth, and these were used to supplement observations from the fresh material. It was found that the endocranial measurements were relatively normal, but the bones were very thin, and the air sinuses were poorly developed. The growth of the jaws was severely retarded, the maxilla being more affected than the mandible. Tooth development and eruption and the resorption of the deciduous teeth were all delayed. However, the crown size of the teeth was unaffected except for the third molars. There was often severe malocclusion, which was related to the reduced growth of the jaws and normal-sized teeth and the apparent more forward development of the mandible. There was extensive attrition of both temporary and permanent teeth. Rehabilitation of the undernourished pigs restored the proportions of their skulls and jaws to normal but left many of the abnormalities of the teeth as they were.

47. Some Aspects of Tooth Replacement in Pleurodont Lizards.—*J. S. Cooper, Dental Hospital, University of Bristol.* The lizards under investigation are those of the genus *Lacerta,* which have a pleurodont and polyphydont dentition. The mode of attachment of the teeth makes possible, in dried specimens, direct examination of both functional and developing teeth and the extent of erosion of the bases of the functional teeth. While alternation is a prominent feature in tooth replacement, examination reveals other constant features in the order of replacement. In dried specimens, if the tooth row is divided into groups of five teeth (from the mid-line), the probable order of replacement of the members of a group can be ascertained. This order is essentially the same in every group. One tooth in each group is replaced at about the same time, so that as many teeth are erupting at one time as there are groups of five teeth in the row. If alternate teeth are examined (as if the row consisted of two overlapping series), the developing successors are seen to be progressively younger from the back toward the front of the row. This descending order of age suggests waves of eruption, passing forward from behind and occupying approximately two groups of five teeth. Hence, considering all the teeth, the same number of waves can be traced in the row as there are groups of five teeth. Evidence in support of this appearance is accumulating from observations on the living animals, which were examined regularly by taking impressions of the teeth. These findings are obviously interrelated, but it is not known which arrangement is the fundamental governing factor in the order of replacement.

48. The Matrix in Caries-resistant Teeth.—*K. Little, Nuffield Department of*

Orthopaedic Surgery, Oxford. Slices of teeth from normally caries-prone subjects and from caries-resistant subjects (from areas with fluorine in the drinking water, natural caries-resistance, arrested caries, and from a caries-free population of 1800 years ago) were treated with a "saturated solution" of calcium phosphate in 5 per cent formic acid. The component of the matrix which normally occurs in the prism cores dissolved from the caries-prone teeth but remained insoluble in the caries-resistant teeth. Under the conditions of the experiment, mineral crystallites showed no indication of chemical attack.

49. Genetic and Chromosomal Analysis of a New Oral Syndrome Consisting of Pseudo-Clefts of the Tongue, Palate, Upper Lip, Familial Trembling, Hypoplasia of Alar Cartilages, and Oligophrenia.—*Robert J. Gorlin, University of Minnesota, U.S.A.* An account of this syndrome is included in the report of thirty-ninth International Association for Dental Research meeting held in Boston, Massachusetts, 1961.

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Succinic Dehydrogenase Activity in Odontoblasts

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Orban (*J.A.D.A.*, **16**:1547, 1929), Bevelander (*Anat. Rec.*, **81**:79, 1941), and Symons (*J. Anat.*, **90**:117, 1956) attributed dentin collagen fiber formation to subodontoblasts. However, morphologic evidence from electron microscopy indicates that odontoblasts synthesize most of the dentin collagen, Korff's fibers forming a relatively small proportion (Nylen and Scott, *Pub. Health Serv. Pub.*, No. 613, 1958).

Recently, Burstone (*Ann. N.Y. Acad. Sci.*, **85**:431, 1960) has reported that the "odontoblastic zone" shows a slight staining for succinic dehydrogenase activity after incubation of frozen sections in tetrazolium-succinate. Dehydrogenase activity is related to ATP synthesis and therefore can be interpreted as an approximate measure of the rate of protein synthesis. Because this method appears to offer more direct evidence of collagen synthesis by cells than purely morphologic methods, we have re-examined the tetrazolium-succinate staining of odontoblasts and subodontoblasts. The technique used was a modification of a method which localizes the enzyme activity within millimicrons (Yaeger, *Exptl. Cell Res.*, **22**:493, 1961).

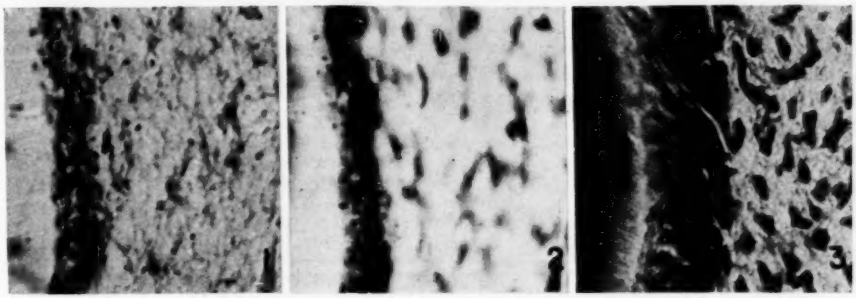

FIG. 1.—*1*, Mouse molar tooth bud. Frozen-dried, stained with tetrazolium-succinate. *2*, Same section as in *1*, stained with hematoxylin and eosin. *3*, Rat molar tooth bud. Fixed in Zenker-formol, stained with hematoxylin and eosin. Dentin at left. (Orig. Mag. approx. ×750.)

Molar tooth germs from 7-day-old mice were frozen in isopentane chilled in liquid nitrogen. The tissues were dehydrated in vacuo at −30° C., infiltrated in vacuo with a solution containing 1 mg/ml Nitro BT* in 0.1 M sodium succinate in 0.1 M phosphate buffer at pH 7.4 and incubated at 37° C. for 15 minutes. In this medium, Nitro BT was reduced to its insoluble, blue formazan by succinic dehydrogenase activity. The tissues were then decalcified in sodium citrate-formic acid in 10 per cent formalin, dehydrated in tertiary butyl alcohol, imbedded in paraffin, and sectioned. Sections were deparaffinized in petroleum ether, stained with hematoxylin and eosin, and re-photographed.

Blue granules indicating the sites of succinic dehydrogenase activity were found concentrated in the odontoblasts, while only occasional granules were seen in subodontoblasts (Fig. 1). These granules were still present after hematoxylin and eosin staining (*2*); *3* is a hematoxylin and eosin-stained section from similar tissue fixed in Zenker formol.

The presence of succinic dehydrogenase activity in odontoblasts and its almost complete absence from subodontoblasts demonstrated by this method strongly suggest that circumpulpul dentin collagen is synthesized almost entirely by odontoblasts.

This investigation was supported by U.S.P.H.S. research grant A-3220 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

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The Inhibition and Stimulation of Oral Lactobacilli by Caries-immune and Caries-susceptible Salivas

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While investigating the bacterial inhibitory properties of human saliva by an agar well-plate technique, it was found that the growth of some species of oral lactobacilli were stimulated by both caries-immune and caries-susceptible salivas while other species were inhibited. Strains of the two types of organisms were isolated and preliminary classification studies made, using for identification the criteria of Rogosa and Sharpe (*J. Appl. Bacteriol.*, **22**:329, 1959). The strains (No. 14 series) which are inhibited by human saliva have been tentatively classed as lactobacilli of the subgenus *Streptobacterium*. They are characterized as Gram-positive, non-sporulating, aciduric, homofermentative rods which grow readily at 15° C. The strains (No. 12 series) which are stimulated by human saliva are Gram-positive, non-sporulating, aciduric, heterofermentative rods which do not grow at 15° C. They have been classed as lactobacilli of the subgenus *Betabacterium*.

To study the effects of saliva on the growth of lactobacilli, whole saliva was collected by paraffin stimulation, pooled, and frozen for storage. Before use, the saliva was thawed and filtered by suction, first, through two layers of Whatman No. 1 filter paper and then through Selas porcelain filters. The filtrate was concentrated by pervaporation and then dialyzed against distilled water. The dialyzate and the non-dialyzable portion of saliva were adjusted to the volume of the original saliva sample. The two solutions were then added separately and in combination to wells cut into plates of the selective agar medium of Rogosa and Sharpe which had been seeded with the various isolated strains of oral lactobacilli. The plates were incubated for 48 hours at 5° C. and then at 37° C. for 48 hours.

Test Organism	Initial Optical Density	Optical Density at 24 Hours*		
		Saline	Immune	Susceptible
Inhibited strain (14B).....	0.102±0.005	0.374	0.109	0.323
Stimulated strain (12A).....	0.094±0.005	0.112	0.143	0.158

* Medium: Saline suspension of cells added to a solution of 4 ml. of saliva (diluted to one-fifth original concentration), or saline, plus 6 ml. of the selective medium of Rogosa and Sharpe. Incubation at room temperature.

Growth of the No. 14 series organisms was inhibited around the wells that contained both the dialyzable and non-dialyzable factors but not around the wells that contained either factor alone. No inhibition occurred when the undialyzed saliva filtrate was heated at 75° C. for 8 minutes. The inhibition appears to be identical with the inhibitory effect described by Kerr and Dogon (Studies on the Inhibition of *L. casei* by Parotid Secretion, *J. D. Res.*, **40**:722, 1961 [abstr.]).

Growth of the No. 12 series organisms was stimulated around those wells that contained both the dialyzable and the non-dialyzable factors, regardless of whether or not either or both of the factors had been heated at 75° C. for 8 minutes prior to their recombination. No stimulation occurred around the wells that contained either factor alone.

The effects of caries-immune and caries-susceptible salivas on the growth of lactobacilli were measured by a turbidimetric method. The filtrate described above was lyophilized and the residue dissolved in saline to 5 times the original volume of saliva for testing. Initial results showed that saliva from caries-susceptible subjects had a greater stimulating effect on lactobacillus strains of the No. 12 series than did equivalent volumes of saliva from caries-immune subjects. Conversely, saliva from immune subjects showed greater inhibition of lactobacillus strains of the No. 14 series than did equivalent volumes of saliva from caries-susceptible subjects. The results of a typical experiment are given in the table.

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Improved Starch-Gel Electrophoretic Resolution of Parotid-Fluid Protein

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In earlier investigations the electrophoretic resolution of parotid-fluid protein in starch gel at alkaline pH was affected by amylatic action in the medium (K. C. Hoerman and V. J. Berzinskas, *J. Lab. Clin. Med.*, **53**: 64, 1959). Although satisfactory comparisons with boundary electrophoretic patterns could be made, the major drawback of the procedure was that the physical properties of the gels after electrophoresis were poor and handling became tedious. Notwithstanding, starch-gel supports are considered superior to all other solid media used in zone electrophoresis (O. Smithies, *Advances in Protein Chem.*, **14**:65, 1959). We have now developed a starch-gel system free of the limiting action of parotid amylase and in which the intrinsic and probable extrinsic protein fractions of parotid protein may be clearly resolved.

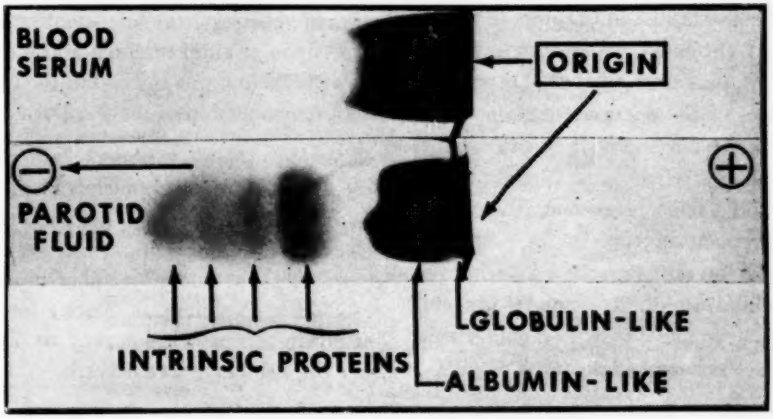

FIG. 1.—Photograph of stained starch gel after electrophoresis, showing comparison of blood serum and parotid-fluid protein migration and the distinct separation of intrinsic from probable extrinsic parotid proteins. Electrophoresis in formic acid, pH 2.0, 6 volts/cm (25 ma), 16 hours.

Twenty-five ml. of parotid fluid were collected with a Lashley parotid cup. The protein content was 2.1 mg/ml (Biuret reaction). The fluid was dialyzed against PVP (polyvinylpyrrolidone, 90 per cent, *w/v*) in the cold (4° C.) with a continuous flow of distilled water into the dialysis bag. After 16 hours, four volumes of cold acetone were added to the salt-free parotid fluid, and the precipitate was collected by centrifugation and dried *in vacuo* over NaOH pellets. Twenty mg. of the parotid protein were dissolved in 0.3 ml. of 0.75 M formic acid, pH 2.0. The protein was submitted to electrophoresis in starch gel composed of 12 gm. of potato starch per 100 ml. of formic acid solution. This formic acid solution was used as bridge electrolyte. Electrophoresis was for 16 hours at 120 volts (25 ma). Gels were stained with Amido-Schwartz dye. Migration was toward the cathode because of the low pH. The four intrinsic parotid proteins were sharply separated from the suspected extrinsic (serumal) proteins. These intrinsic parotid proteins migrated at faster rates because they were strongly cationic, basic in nature, and probably of low molecular weight. All trailing—a consistent feature of previous efforts to separate parotid proteins in solid medium (I. D. Mandel and S. A. Ellison, *Arch. Oral Biol.*, **3**:77, 1961; Hoerman and Berzinskas, *op. cit.*)—was eliminated in the present electrophoretic system. It was noteworthy that albumin-like and globulin-like proteins were also distinctly resolved. Direct characterization of the various parotid fluid proteins isolated from similar starch-gel experiments has been undertaken.

This investigation was supported in part by U.S.P.H.S. research grant D-602 from the National Institute of Dental Research, National Institutes of Health.

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